versus 54.4%). Nearly half (15, 48.4%) of all dengue patients met criteria for influenza (i.e., fever with cough or sore throat), and the majority (78.7%) of influenza patients met criteria for dengue fever. Dengue patients were more likely than influenza patients to have bleeding (80.6% vs. 26.5%), rash (38.7% vs. 8.8%), and positive tourniquet test (51.6% vs. 18.1%). Mean platelet count was 74,484 ± 58,000 for dengue patients and 189,639 ± 57,400 for influenza patients while mean white blood cell count was 3,400 ± 1,400 and 5,800 ± 2,800, respectively. Clinical diagnosis can be especially difficult when outbreaks of other AFI occur during dengue season. Our findings highlight the focal nature of dengue outbreaks and suggest that physician notification to public health officials should be encouraged. With many dengue patients meeting the case definition for influenza and vice versa, complete blood count and tourniquet test may be useful to differentiate dengue from other AFIs.

467

SEVERE CO-INFECTIONS OF DENGUE AND PANDEMIC INFLUENZA A H1N1 VIRUSES

Aubree Gordon[1], Maria Angeles Pérez[2], Felix Sanchez[2], Federico Narvaez[2], Gamaliel Gutierrez[3], Oscar Ortega[3], Andrea Nuñez[4], Eva Harris[1], Angel Balmaseda[4]

[1]Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, Berkeley, CA, United States, [2]Hospital Infantil Manuel Jesús de Rivera, Managua, Nicaragua, [3]Sustainable Sciences Institute, Managua, Nicaragua, [4]Departamento de Virología, Centro Nacional de Diagnóstico y Referencia, Ministerio de Salud, Managua, Nicaragua

Dengue and influenza are both acute-onset viral illnesses that can initially present with similar symptoms. Epidemics of influenza and dengue generally do not overlap in Nicaragua, and virus co-infections have not been documented. However, in September 2009, simultaneous high rates of transmission of pandemic influenza and dengue in Nicaragua resulted in co-infections. Here we report on four hospitalized patients with dengue-influenza virus co-infections. All patients were RT-PCR positive for dengue virus serotype 3 and for pandemic influenza A H1N1. Clinical findings at presentation ranged from influenza-like illness to severe dengue. The clinical progression of the infections varied by case, but all developed classic dengue symptoms and had interstitial and/or alveolar infiltrates. Three cases required intensive care including mechanical ventilation, and one was fatal. All of the cases requiring mechanical ventilation had asthma, and the fatal case was also obese. Thus, dengue-influenza virus co-infections may lead to severe disease and can be fatal. Due to the varied clinical presentation and difficulties differentiating dengue-influenza virus co-infections from single infections, especially early after symptom onset, it is advisable that testing for both viruses be performed when they are co-circulating.

468

DETERMINANTS OF RISK FOR CARDIOVASCULAR SHOCK AND MORTALITY IN HOSPITALIZED DENGUE PATIENTS IN HO CHI MINH CITY, VIETNAM

Katherine Anders[1], Minh Nguyet Nguyen[1], Vinh Chau V. Nguyen[2], Thanh Hung Nguyen[3], Thi Thuy Tran[4], Bich Lien Le[3], Jeremy Farrar[1], Bridget Wills[1], Tinh Hien Tran[1], Cameron Simmons[1]

[1]Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam, [2]Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam, [3]Children's Hospital Number 1, Ho Chi Minh City, Vietnam, [4]Children's Hospital Number 2, Ho Chi Minh City, Vietnam

Dengue represents a growing global public health challenge. Understanding trends in disease burden and epidemiology is important for vector control, allocation of health services and planning the introduction of vaccines and therapeutic drugs. We analysed clinical and demographic trends in the dengue case burden in Ho Chi Minh City, Vietnam, between 1996 and 2009, and assessed risk factors for dengue shock syndrome (DSS) and mortality among 102,494 dengue patients admitted between 2000 - 2009. The dengue caseload across the three hospitals increased over the study period, to a peak in excess of 20,000 cases in 2008. Adults represented an increasing proportion of cases over time. The vast majority (13,595/14,079; 96.6%) of patients with DSS were children, with those aged 6 - 10 at higher risk of DSS than younger or older children. In contrast, the risk of mortality was highest in younger children and decreased with age (OR 0.52, 95% CI 0.36 - 0.75 in 6 - 10 year olds and OR 0.27, 95% CI 0.16 - 0.44 in 11 - 15 year olds, compared with 1 - 5 year olds). Overall mortality was low (0.20%) and progressively decreased during the study period (estimated change per year = -0.04%, 95% CI -0.06% - -0.02%). Males were overrepresented among dengue cases, suggesting a gender difference in healthcare seeking behaviour and/or susceptibility to disease. Strikingly however girls had a higher risk of DSS (OR 1.19, 95% CI 1.14 - 1.24) and death (OR 1.57, 95% CI 1.14 - 2.17) than boys. This hospital caseload indicates a startlingly high dengue disease burden in Ho Chi Minh City, with at least 1 in 400 people and 1 in 140 children admitted to one of the three study hospitals with dengue in 2008. In conclusion, the risk of DSS and death is highest in young female children. Young children are at greatest risk of death and this population should be targeted in clinical trials of dengue vaccines and therapeutics. The increased risk of severe outcomes in girls warrants further attention both in studies of dengue pathogenesis and of health-seeking behaviour, and in clinical care.

469

OLDER AGE IS A RISK FACTOR FOR SYMPTOMATIC DENGUE VIRUS INFECTION IN NICARAGUAN CHILDREN

Aubree Gordon[1], Guillermina Kuan[2], Oscar Ortega[3], Juan Carlos Matute[4], Juan Carlos Mercado[4], Josefina Coloma[1], William Aviles[3], Angel Balmaseda[5], Eva Harris[1]

[1]University of California, Berkeley, Berkeley, CA, United States, [2]Socrates Flores Vivas Health Center, Ministry of Health, Managua, Nicaragua, [3]Sustainable Sciences Institute, Managua, Nicaragua, [4]National Center for Diagnosis and Reference, Managua, Nicaragua, [5]National Center for Diagnosis and Reference, Ministry of Health, Managua, Nicaragua

The Nicaraguan Pediatric Dengue Cohort Study is a prospective cohort study, established in August 2004, to examine the incidence and clinical manifestations of dengue virus (DENV) infection in children 2-14 years old in Managua, Nicaragua. Children were enrolled prospectively, with yearly participation of 3,693-3,795 children. Participants are encouraged to come to the study Health Center at first sign of illness and all medical care is provided free-of-charge. Participants with suspected dengue or undifferentiated fever are tested for dengue by RT-PCR, virus isolation, and serological assays. Additionally, yearly blood samples from all cohort members are collected to determine the incidence of inapparent DENV infection. Univariate and multivariable generalized estimating equations (GEE) with a Poisson model were used to examine risk factors for symptomatic disease given DENV infection. Variables included in the multivariate models were: year of study, immune status, sex, and age. In the first 4 years of the study, 159 acute dengue cases and 9 DHF/DSS cases were detected, yielding an incidence rate of 11.2 (95% CI 9.6, 13.1) acute dengue cases and 0.65 DHF/DSS cases per 1000 person-years. During the same period, 1,047 DENV infections (symptomatic and inapparent) were detected, yielding an incidence of 78.9 (95% CI 74.2, 83.8) DENV infections per 1000 person-years. The incidence of cases and infections as well as the ratio of cases to infections varied substantially year-to-year. Incidence of cases varied markedly by age, with the highest incidence rate of symptomatic dengue in 10 year-olds. In contrast, the incidence of DENV infection was more constant across ages, with the highest incidence observed in the youngest one-year age groups. In multivariable models, age group (9-12 years old) was a significant predictor of symptomatic disease given infection (incidence rate ratio (IRR) 1.9; 95% CI 1.2-2.9), but immune status was not (IRR 1.3; 95% CI 0.9-1.8). Stratifying by immune status revealed that age is an important risk factor for developing symptomatic infection among primary DENV infections (IRR 4.0; 95% CI

1.7-9.7). Multiple children experienced two or more DENV infections. We are currently examining the effect of nutrition on risk for symptomatic or severe disease and investigating serial DENV infections in the cohort. This study is providing critical data on the epidemiology and transmission of dengue in the Americas.

470

A LONGITUDINAL ANALYSIS OF MATERNAL DENGUE ANTIBODY KINETICS AMONG INFANTS IN BANGKOK

Willem G. Van Panhuis[1], Christine Luxemburger[2], Krisana Pengsaa[3], Kriengsak Limkittikul[3], Arunee Sabchareon[3], Derek A. Cummings[4], Jean Lang[2], Anna P. Durbin[4]

[1]University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA, United States, [2]Sanofi Pasteur, Lyon, France, [3]Department of Tropical Pediatrics, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, [4]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Maternal dengue antibodies are important factors for dengue pathogenesis and vaccine efficacy in infants. Previous studies have estimated the proportion of infants with detectable antibody levels and single, monophasic decline rates. These rates have been used to extrapolate antibody levels at birth to estimate values at later ages. No longitudinal analysis of the heterogeneity in antibody decline between and within infants has ever been conducted to provide more in depth knowledge on underlying patterns and determinants. Data from a previous cohort study of 140 infants in Bangkok were used to estimate serotype-specific decline rates of maternal, neutralizing dengue antibodies and social-economic determinants. Longitudinal regression methods were used to model average decline rates for different age intervals and to detect atypical patterns that were significantly different from the average pattern in the rest of the cohort. Antibody decline rates between birth and 3 months of age ranged from 51 to 58% per month. For DENV-1, 2 and 4, these rates were significantly different from rates between 3-9 months: 36, 38 and 13% respectively. Decline rates after 9 months for these serotypes were not significantly different from zero. For DENV-3, only two age intervals were identified with a decline of 36% per month between 3-12 months. For DENV-1, a significantly lower decline rate was found for infants with atypical patterns (17%). For DENV-4, a faster decline rate was found in such infants (26%). This is the first study that applied longitudinal methods to estimate maternal dengue antibody decline rates. Single, monophasic rates have been used previously, but based on our data we suggest using age specific decline rates to improve the accuracy of extrapolations. This could be of great use to studies on the optimal age of vaccination and dengue pathogenesis. Atypical decline patterns were found that may imply asymptomatic DENV infections in infancy, which could have implications for the response to vaccination in this age group.

471

IDENTIFICATION AND CHARACTERIZATION OF NOVEL HUMAN ANTI-DENV-2 MONOCLONAL ANTIBODIES THAT DO NOT TARGET DOMAIN III OF THE DENV-2 ENVELOPE GLYCOPROTEIN

William Messer[1], Ruklanthi de Alwis[2], Quang Pham[3], Jeremy Huynh[4], Martina Betramello[5], Federica Sallustro[5], Antonio Lanzavecchia[5], Ralph S. Baric[4], Aravinda de Silva[2]

[1]University of North Carolina School of Medicine, Department of Medicine, Chapel Hill, NC, United States, [2]University of North Carolina School of Medicine, Department of Microbiology and Immunology, Chapel Hill, NC, United States, [3]University of North Carolina School of Medicine, Chapel Hill, NC, United States, [4]University of North Carolina Gillings School of Global Public Health, Chapel Hill, NC, United States, [5]Institute for Research in Biomedicine, Bellinzona, Switzerland

Despite the worldwide importance of dengue virus (DENV) as a pathogen, the basis of both protective immunity and pathogenesis in the human host remains incompletely understood. The principal target of the neutralizing human antibody response is the DENV envelope glycoprotein (E). Epitopes on the E glycoprotein may also play a role in enhancing viral infection through the attachment of cross-reactive, non-neutralizing heterotypic antibodies. The E glycoprotein consists of 3 domains designated EDI, EDII and EDIII. Mouse antibodies that strongly neutralize DENV mainly bind to EDIII. We have previously demonstrated that, unlike mice, humans exposed to DENV mainly have neutralizing antibodies that bind to epitopes outside EDIII, presumably on EDI or II. The goal of this study was to use human mAbs to map neutralizing epitopes on EDI and EDII. Human mAbs were isolated from memory B cells of a donor with a history of DENV2 infection. To identify the location of epitopes on EDI and II, DENV was passaged serially in the presence of excess neutralizing antibody to select for escape mutants. Neutralizing mAb escape was confirmed by plaque reduction neutralization test. Antibody escape mutants were plaque-purified and their E genes sequenced and mapped onto the crystal structure of the E glycoprotein dimer. These studies demonstrate that naturally infected persons develop memory B-cells that produce neutralizing mAbs directed to epitopes on EDI and II of DENV.

472

DENGUE VIRUS-SPECIFIC T CELL RESPONSES MORE THAN SIXTY YEARS AFTER INFECTION

Alexandra Grurary[1], Janet Meeks[1], Munkhzul Sukhbaatar[1], Allison Imrie[2]

[1]University of Hawaii at Manoa, Honolulu, HI, United States, [2]University of Western Australia, Perth, Australia

We assessed long term dengue virus (DENV)-specific memory T cell responses in individuals exposed more than 60 years previously, during the 1940s Pacific DENV epidemics. We compared these data to responses in 10 individuals infected in 2001 during a DENV-1 epidemic in Hawaii, and to 10 control subjects with no serological evidence of prior DENV infection. PBMC were collected from 7 individuals more than 60 years after they experienced a dengue-like illness in Hawaii or in the Pacific. PRNT90 analysis confirmed previous exposure to DENV-1 in 4 of 7 individuals (reciprocal titres > 1:160) with low level responses (1:10) in one other subject. Proliferative responses to DENV-1, DENV-2, DENV-3, and DENV-4, and memory markers, were assessed by FACS, and IFN-gamma responses measured by ELISPOT and ICS. DENV-specific CD4+ T cell responses were long-lived and detectable after 60 years in most subjects, whereas DENV-specific CD8+ responses declined over time (Multiparameter ANOVA; $p < 0.001$) and were not measurable in 2 subjects with robust CD4 responses. DENV-1-specific memory T cells were primarily of the CD4+ central memory CD45RA-CCR7+CD62L+ phenotype in contrast to the predominantly CD8+ effector CD45RA-CCR7-CD62L- phenotype of the 2001 cohort up to 5 years after infection. DENV1-specific memory T cells were highly cross-reactive with DENV-2, DENV-3, and/or DENV-4 in both groups, and to a higher degree in the 1940s group. We have identified and characterized DENV-specific immune responses more than six decades after infection. These findings may contribute to our understanding of DENV pathogenesis, and to the design of safe and effective dengue vaccines.

473

NO EVIDENCE THAT ARTEMISININ-RESISTANT MALARIA HAS SPREAD TO SOUTH ASIA

Peter Starzengruber[1], Hans-Peter Fuehrer[1], Paul Swoboda[1], Verena Hofecker[1], Anja Siedl[1], Markus Fally[1], Wasif A. Khan[2], Emran B. Yunus[3], Shah M. Hossain[4], Pascal Ringwald[5], Harald Noedl[1]

[1]Medical University of Vienna, Vienna, Austria, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [3]Chittagong Medical College, Chittagong, Bangladesh, [4]Ministry of Health and Family Welfare, Dhaka, Bangladesh, [5]World Health Organization, Global Malaria Programme, Geneva, Switzerland

Virtually all malaria-endemic countries have officially adopted artemisinin-based combination therapies (ACTs) for the treatment of uncomplicated *falciparum* malaria. However, recent data indicate that the first cases of genuine artemisinin resistance have already emerged along the Thai-Cambodien border.

We conducted an open-label, randomized, controlled 42-day clinical trial in southeastern Bangladesh to investigate the potential spread of clinical artemisinin resistance from SE-Asia. A total of 126 uncomplicated *falciparum* malaria patients were randomized to one of 3 treatment arms (artesunate monotherapy with 2 or 4 mg/kg/day once daily for 7 days or quinine plus doxycycline TID). Treatment response and safety parameters were closely monitored throughout the study. In vitro drug sensitivity was assessed by HRP2 assay and samples for genotyping were collected on admission and in case of re-emergence of parasitemia. Only cases fulfilling all of the following criteria were defined as being artemisinin-resistant: recrudescence during follow up; prolonged PCT; exclusion of re-infection; pharmacokinetic parameters confirming adequate drug levels; in vitro data or genetic makers indicating reduced drug susceptibility. The 42-day cure rates in the artesunate monotherapy (2 and 4 mg/kg) and quinine/doxycyline arms were 97.8%, 97.7% and 100%, respectively. A single case of re-infection was seen in each of the artesunate arms, not a single case of recrudescence was observed during this trial. No differences in median PCT and FCT were found between the 2 artesunate arms (29.8 hrs and 17.9 hrs vs. 29.5 hrs and 19.1 hrs). No serious adverse events were observed. The parasite phenotype seen in Bangladesh is likely to be representative of Asian Plasmodium *falciparum* populations before the introduction of artemisinins. Not a single case fulfilled our criteria of artemisinin resistance. PCTs were considerably shorter and in vitro results indicate significantly higher susceptibility to artemisinins as compared to SE-Asia. There was also no indication of compromised intrinsic drug sensitivity to artemisinins and treatment response was not dose-dependent.

474

PREVALENCE AND SELECTION OF *PLASMODIUM FALCIPARUM* DRUG RESISTANCE MOLECULAR MARKERS UNDER INTERMITTENT PREVENTIVE THERAPY IN BURKINA FASO

Some Anyirekun Fabrice

IRSS-BOBO, Bobo-dioulasso, Burkina Faso

Single nucleotide polymorphisms (SNPs) in the *P. falciparum pfcrt*, *pfmdr1*, *pfdhfr* and *pfdhps* genes are associated with decreased response to aminoquinoline and antifolate antimalarials and have been shown to be selected by use of these drugs. The degree of selection by intermittent preventive therapy (IPT) regimens is unknown. We assessed the baseline prevalence and selection of common SNPs by IPT in children in Bobo-Dioulasso, Burkina Faso. We studied 1500 children (aged 3-59 months) randomized to receive monthly dihydroartemisinin-piperaquine (DP) or amodiaquine-sulfadoxine/pyrimethamine (AQ/SP) for 3 months during the malaria transmission season in 2009. The efficacy, safety and tolerability of DP vs. AQ/SP will be described elsewhere. For random samples of 120 children from each arm of the study and for 120 of 250 untreated controls we evaluated the prevalence of key resistance-mediating SNPs. We then assessed the prevalence of the same SNPs in samples collected in November, 1 month after the third of 3 monthly treatments with DP or AQ/SP.Before therapy malaria prevalence was 48.1% based on microscopy and 72.5% measured by PCR. Prevalences of SNPs before therapy were 68.2% (178/261) for *Pfcrt* 76T: 24.9% (65/261), 56.3% (147/261) and 8.0% (21/261) for *Pfmdr1* 86Y, 184F and 1246Y, respectively; 58.6% (153/261), 54.8% (143/261), and 55.17% (144/261) for *Pfdhfr* 51I, 59R and 108N, respectively; and 33.7% (88/261) and 57.47% (157/261) for *Pfdhps* 436S and 437G. The SNPs *of Pfmdr1* 1034C and 1042D; *Pfdhfr* 164L; and *Pfdhps* 540E were not seen. SNP prevalences after three monthly treatmens are currently being analyzed. Our results indicate high prevalence of key resistance-mediating polymorphisms. Associations between the prevalence of these SNPs and IPT will be assessed.

475

UNDERRATING MOSQUITOES YET AGAIN: THE EVASION OF SURVEILLANCE BY *PLASMODIUM FALCIPARUM* DRUG RESISTANCE MUTANTS

Sungano Mharakurwa[1], Mulenga Musapa[1], Clive Shiff[2], Philip E. Thuma[1]

[1]The Malaria Institute at Macha, Choma, Zambia, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

By definition, "malaria" was an initial underrating of mosquitoes, ascribing the deadly disease to "bad air". Ironically, present-day surveillance for drug-resistant *Plasmodium falciparum* mutants is primarily based on genotyping microscopy-positive human infections and presumed representative of the surveyed areas. Genotypes in the definitive mosquito host are seldom examined, despite field evidence of association between mosquito control and drug-resistant *P. falciparum* prevalence. In the current study, we captured 796 *Anopheles arabiensis* vector mosquitoes from sleeping rooms of a representative sample of 2279 human residents in Southern Zambia. We examined the cross-sectional composition of *P. falciparum* antifolate resistance polymorphisms in both human and mosquito infections using PCR, allele-specific restriction enzyme digestion and DNA sequencing confirmations. High levels of pyrimethamine resistance mutants were found in human *P. falciparum* infections, with nearly saturated S108N (92.7%) and considerably prevalent N51I (81.5%) and C59R (58.5%). In contrast, the odds of these mutants were up to 101-fold lower in the mosquito phase (OR [95% CI]: 101.3 [34.34 - 299.03], $p < 0.001$). Mosquitoes, instead, exhibited high prevalence of cycloguanil resistance S108T/A16V mutants, which are currently considered rare or absent from natural *P. falciparum* infections, especially in Africa. We initially did not detect these mutants in humans but subsequently found them among submicroscopic infections. Cycloguanil has not been used in the area. One mosquito mid-gut infection carried the rare I164L mutant, while another bore a hitherto undescribed I164R variant. Our data demonstrate that *P. falciparum* exhibits different antifolate resistance allele compositions in human and mosquito hosts, presumably reflecting contrasted drug and (or) immune selection. We show that by dint of such host-dependent distribution, *P. falciparum* mutants apparently evade current surveillance for resistance alleles. An unnoticed role of mosquitoes in drug resistance epidemiology is presented.

476

TARGET GENE DUPLICATION IN ARMD *PLASMODIUM FALCIPARUM* ACQUIRING DRUG RESISTANCE

Jennifer L. Guler[1], Daniel L. Freeman[1], Napawan Ponmee[1], John White[1], Ramesh Gujjar[1], Alka Marwaha[1], Margaret A. Phillips[2], Pradipsinh K. Rathod[1]

[1]University of Washington, Seattle, WA, United States, [2]University of Texas Southwestern Medical Center, Dallas, TX, United States

Drug resistance to commonly used antimalarials has produced major barriers to the treatment of *Plasmodium* infections. Some parasites from Southeast Asia exhibit the ARMD phenotype, an enhanced ability to develop resistance to new, unrelated antimalarials *(*as reported previously). Recently, we selected for 5-fold stable resistance to a novel dihydroorotate dehydrogenase inhibitor (DSM1; as reported previously) in ARMD Dd2 parasites. While direct sequencing revealed no mutations in the target gene, comparative genomic hybridization showed a 34-95kb amplification event at the dihydroorotate dehydrogenase locus in four independent DSM1-resistant clones. Quantitative PCR and expression analysis confirmed a two- to three-fold increase in copy number of the target and surrounding genes. Theoretically, amplification events up to 100kb would allow for coverage of the entire 25Mb *Plasmodium* genome with as few as 250 parasites. We propose that this process serves as a critical, early event in the accelerated acquisition of resistance and that extra copies of the target gene may facilitate the accumulation of mutations in a way that is missed by conventional sequencing. Adaptive amplification followed by mutagenesis could be a general strategy that ARMD parasites use to survive and evolve resistance during lethal selection.

477

MEASURING MALARIA PARASITE CLEARANCE USING REAL-TIME PCR ON BLOOD-SPOT DERIVED PARASITE DNA

Khalid B. Beshir[1], Teun Bousema[1], Rachel Hallet[1], Spencer Polley[2], Colin Sutherland[1]

[1]London School of Hygiene and Tropical Medicine, London, United Kingdom, [2]Hospital for Tropical Diseases, London, United Kingdom

Early detection of slow clearing malaria parasites enables adequate countermeasures to be taken to prevent the emergence of drug resistance. One way of achieving this is by measuring change in peripheral parasitemia in a sequence of samples taken after treatment, and currently this requires microscopic examination of blood films. However, microscopic estimation of parasite clearance time requires considerable expertise; false negatives can easily be read especially at low-level parasitemia; it is labour intensive and suffers from low throughput. We report the development and use of a new multi-plex quantitative PCR (qPCR) assay to measure parasite clearance after treatment. The method is based on the Delta Delta Cycle Threshold (ΔΔCT) method to estimate the relative abundance of two amplification targets, one of parasite origin and one of human origin. The assay was tested on patient blood samples taken before and after treatment and the results were compared to microscopy. Field samples were also evaluated, and individuals harbouring malaria parasites with slower responses to artemether-lumefantrine were successfully identified. This new method is quicker, less laborious, more sensitive and requires less training compared to microscopy. We are planning to incorporate the assay as an endpoint in large scale clinical trials of Artemisinin Combination Therapy (ACT) efficacy in Africa. Filter-paper based identification of slow responding parasites is a valuable surveillance tool for early warning of the emergence of drug resistance.

478

DEFINING THE ROLE OF MUTATIONS IN *PLASMODIUM VIVAX* DIHYDROFOLATE REDUCTASE-THYMIDYLATE SYNTHASE USING A *PLASMODIUM FALCIPARUM* EXPRESSION SYSTEM

Alyson M. Auliff[1], John H. Adams[2], Michael T. O'Neil[3], Qin Cheng[1]

[1]Australian Army Malaria Institute, Enoggera, Australia, [2]Department of Global Health, University of South Florida, Tampa, FL, United States, [3]Division of Experimental Therapeutics, Walter Reed Army Institute of Reseach, Silver Spring, MD, United States

With the high prevalence of *Plasmodium vivax* resistance to antifolates throughout Australasia, it is critical to understand the determinants for resistance and for development of new treatments. Like *P. falciparum*, resistance to antifolates such as pyrimethamine and cycloguanil in *P. vivax*, are caused by point mutations within the parasites dihydrofolate reducatase (DHFR)-thymidylate synthase genes. However several unique mutations have been reported in *P. vivax* DHFR and their roles in resistance to classic and novel antifolates are not entirely clear. We have assessed the *in vitro* expression of the *P. vivax* wild- type and various mutant dhfr alleles using both episomal and piggyBac transposon integrated *P. falciparum* expression systems and compared the effect of these alleles to susceptibility to antifolates. We show that the *P. falciparum* parasites transfected with wild-type pvdhfr, in both expression systems, is as susceptible to classic and novel antifolates as the *P. falciparum* with wild-type pfdhfr, while *P. falciparum* parasites transfected with mutant pvdhfr are as resistant to classic antifolates as mutant *P. falciparum* and are notably more resistant to a novel antifolate drug WR99210. Our results show that the expression of pvdhfr alleles in *P. falciparum*, a closely related biological system, help identify the role and importance of specific mutations against current and new antifolate treatments and provide a system for the quick assessment of the potency of new antifolate drugs against *P. vivax* with different dfhr alleles.

479

IMPLICATIONS OF THE PHARMACOLOGICAL PROFILE OF ACT FOR FUTURE TREATMENT EFFICACY

Zulfiqarali Gulamhussein Premji

Muhumbili University of Health Sciences, Dar es Salaam, United Republic of Tanzania

We examine the specific pharmacological profiles of the different artemisinin-based combination therapies (ACT) and discuss the implications of these properties on their suitability for malaria treatment now and in the future. The half-life of the artemisinin partner drug has a significant effect on the efficacy of ACT and the potential for development of resistance. A shorter half-life may not eliminate parasites, while a longer half-life may expose parasites to sub-therapeutic drug levels, increasing the risk of resistance development. The emergence of resistance to a companion drug leaves the artemisinin derivative exposed as an unprotected monotherapy, which may jeopardize the longevity of the whole ACT class. With partner drugs that have a relatively short terminal half-life, such as lumefantrine, the risk for the emergence of resistance is lower than other agents such as mefloquine. However, a longer half-life may protect the patient against re-infection for longer. An increase in the development of cross-resistance between chloroquine and companion drugs bearing a structural similarity (artesunate and piperaquine) is also a cause for concern. While a once-daily dosing schedule is more likely to encourage compliance, a twice-daily schedule maintains the blood concentration of the artemisinin derivative above the minimum effective concentration for at least two asexual parasite lifecycles. This ensures that parasites are exposed to high levels of artemisinin derivative at the point in their lifecycle when they are most susceptible to antimalarials. In addition, a progressive increase in concentration of the partner drug means that any residual parasites continue to be exposed to high drug levels, reducing recrudescence. With the artemisinin derivatives being the

only antimalarials to which parasite resistance has not yet been reported in Africa, consideration of pharmacological factors will be important in preserving their effectiveness.

480

MULTIPLEX DIAGNOSTIC PROTOCOL FOR ENTEROPATHOGENS

Mami Taniuchi[1], Jie Liu[1], Jean Gratz[1], Happy Nduma[2], Athanasia Maro[2], Gibson Kibiki[2], Rashidul Haque[3], Jaco Verweij[4], **Eric R. Houpt**[1]

[1]University of Virginia, Charlottesville, VA, United States, [2]Kilimanjaro Christian Medical Centre, Moshi, United Republic of Tanzania, [3]International Centre for Diarrhoeal Diseases and Research, Dhaka, Bangladesh, [4]Leiden University, Leiden, The Netherlands

Detection of enteropathogens typically requires multiple diagnostic modalities with inherently different sensitivities. We sought to develop a pan-PCR approach for enteric viral, bacterial, protozoal, and helminthic targets. First we adapted our real time PCR assays for the major intestinal parasites *Cryptosporidium*, *Giardia*, *Entamoeba histolytica*, *Ascaris*, *Ancylostoma*, *Necator*, and *Strongyloides* into a single protocol involving two multiplex PCR reactions, one with specific primers for the protozoa and one with specific primers for the helminths, after which PCR product is hybridized to beads linked to a specific internal oligonucleotide probe. Detection occurs on a Luminex platform. The assays exhibited equal or better analytic sensitivity than the parent multiplex real-time PCR assays, and yielded an average sensitivity of 91 12% and specificity of 97% 2% for the analytes on 228 clinical specimens from Bangladesh and The Netherlands. Next we developed PCR and RT-PCR assays for enteric viruses, including Norovirus GI and GII, Rotavirus, Astrovirus, Sapovirus and Adenovirus. The assay yielded a tight correlation with Ct values from real time RT-PCR assay (R2=0.94), indicating quantitation, and exhibited > 95% sensitivity and specificity on 229 fecal samples from inpatients with diarrhea from Tanzania. Internal controls for both DNA and RNA are spiked into each fecal sample before nucleic acid extraction to better quantitate by normalizing for the efficiency of nucleic acid extraction and amplification. We are now adding bacterial targets. This multiplex PCR-bead assay will afford sensitive quantitative detection of all major enteropathogens in a single protocol usable for epidemiologic and clinical studies.

481

ROTAVIRUS DIARRHEA IN YOUNG CHILDREN IN BHARATPUR, NEPAL

Ladaporn Bodhidatta[1], Sanjaya Shrestha[2], Sasikorn Silapong[1], Bhola Ram Shrestha[3], Shanti Regmi[3], Orntipa Sethabutr[1], **Carl J. Mason**[1]

[1]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [2]Walter Reed/Armed Forces Research Institute of Medical Sciences Research Unit Nepal, Kathmandu, Nepal, [3]Bharatpur Hospital, Bharatpur, Nepal

Human Rotavirus causes significant morbidity and mortality among children worldwide. Several effective vaccines have become available and can reduce the disease burden and health care costs of rotavirus-specific diarrhea. We conducted a hospital based surveillance of rotavirus diarrhea in Bharatpur Hospital, Bharatpur, Nepal during December 2006 - December 2008 to describe the epidemiology and genotypic distribution of rotavirus, important information for decision-makers on a future vaccine implementation. Stool samples collected from children under 5 years of age with acute diarrhea and non-diarrhea controls were examined for rotavirus by a real time reverse transcriptase polymerase chain reaction (RT-PCR) using primers and probes targeted on VP6 gene. Samples positive for Rotavirus were genotyped using primers targeted on VP7 and VP4 genes to identify 6 G-types (G1-G4, G9 and G12) and 3 P-types (P[4], P[6] and P[8], respectively, by conventional PCR. Rotavirus was detected in 204/598 (34%) of children with diarrhea and 47/597 (8%) of non-diarrhea controls. G12 was the predominant type (69%), followed by G1 (7%), G9 (6%) and G2 (5%). In regard to P-typing, P[8] was predominant (54%) followed by P[6] (29%) and P[4] (7%). The fraction of the major combination of G and P was G12P[8] (36%) followed by G12P[6] (27%). Approximately 13% and 10% could not be characterized by G-typing and P-typing primers, respectively. Results of the sequence analysis will be reported and the emergence of unusual serotypes described. Rotavirus is clearly a significant cause of acute pediatric diarrhea in Nepal with the most common genotypes being G12P[8] and G12P[6].

482

NOROVIRUS INFECTION IN YOUNG CHILDREN IN THAILAND - A MULTICENTER STUDY

Ladaporn Bodhidatta[1], Pimmnapar Neesanant[1], Siriporn Sornsakrin[1], Vorachet Taecharak[2], Krongkaew Supawat[3], Orntipa Sethabutr[1], Carl J. Mason[1]

[1]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [2]Srisangwan Hospital, Maehongsorn Province, Thailand, [3]Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand

Noroviruses (NoVs) are associated with acute viral gastroenteritis in humans worldwide. In Thailand, the epidemiology of NoV has not been well described. Limited prevalence data was previously reported as 14% in hospitalized children in 2002-2004 with most NoV identified as genogroup II. We developed and validated a one step real time Reverse Transcription Polymerase Chain Reaction (rt RT-PCR) assay to detect NoV and distinguish between genotype I and II. This assay was applied to stool samples collected from children with acute gastroenteritis (cases) and children without acute gastroenteritis (controls) in multiple sites in Thailand during October 2004 to December 2006. We tested 3,064 stool samples obtained from 3 month to 5 year old children in four different geographical areas of Thailand. 1,502 samples were collected from diarrhea cases and 1,562 samples were from controls with no recent history of diarrhea. NoV was detected in 211/1,502 (14 %) of cases and in 77/1,562 (5 %) of controls (Odds Ratio = 3.2, 95% Confidence Interval 2.4-4.1). GII was the major genotype accounting for approximately 96% and 92% of all NoV identified from cases and controls, respectively. The prevalence of NoV was higher in children less than 36 months old, during cooler months and at certain geographical areas. Our data suggests that NoV is an emerging important cause of acute gastroenteritis in young children and GII is the predominant genogroup.

483

PROLIFERATION OF CYTOKINE PRODUCING T CELLS IN RESPONSE TO *VIBRIO CHOLERAE* O1 INFECTION OR VACCINATION IN BANGLADESHI ADULTS

Alison L. Kuchta[1], Taibur Rahman[1], Taufiiqur R. Bhuiyan[1], Mohammad Arifuzzaman[1], Rasheduzzaman Rashu[1], Ashraful I. Khan[1], Fahima Chowdhury[1], Amit Saha[1], Edward T. Ryan[2], Stephen B. Calderwood[2], Firdausi Qadri[1], Jason B. Harris[2]

[1]ICDDR,B, Dhaka, Bangladesh, [2]Massachusetts General Hospital, Boston, MA, United States

Vibrio cholerae O1 causes diarrheal disease that may be life-threatening without appropriate treatment. Natural infection confers over 90% protective immunity for 5-7 years, while oral cholera vaccines confer up to 3 years of protection at varying degrees. The CD4+ T-cell responses to V. cholerae O1 infection has not been well characterized but may contribute to the development of longer lasting B-cell responses seen after natural infection. In this study, to examine the profile of cytokine secreting CD4+ T cells following either cholera infection or vaccination, we enrolled V. cholerae O1 infected Bangladeshi adult cholera patients and Dukoral vaccine recipients. CD4+ T-cell responses were assessed by intracellular cytokine staining following stimulation of peripheral blood mononuclear cells with V. cholerae specific antigens. CD4+ T cells producing IFN-γ, IL-

13, IL-10 and IL-17 were analyzed at multiple time points after infection or vaccination and compared with responses in healthy adult controls. In patients, IFN-γ response was significantly higher following stimulation with V. cholerae membrane protein (MP) at the acute (day 2) and early convalescence stages (day 7) of cholera infection. In the same group, there was no increase in IL-13 producing T-cells, suggesting a Th1 type of polarization. In the vaccinated population, a significant increase in both IFN-γ and IL-13 cytokine secreting cells was observed, indicating a mixed Th1 and Th2 type response. An increase in IL-17 secreting CD4+ T cells in response to antigenic stimulation was also observed in both patients and vaccinees. These results show that, in a cholera endemic population, CD4+ T-cell responses are demonstrable at acute and early convalescence stages after infection and following vaccination in adults, and that there are significant differences in the characteristics of patient and vaccinee responses. Further studies are needed to address whether differences in the initial CD4+ T-cell response in patients and vaccinees contribute to differences in the subsequent duration of protective immunity.

484

MUCOSAL IMMUNOLOGIC RESPONSES IN CHOLERA PATIENTS IN BANGLADESH

Taher Uddin[1], **Jason B. Harris**[2], Taufiqur Bhuiyan[1], Tahmina Shirin[1], M. Ikhtear Uddin[1], Ashraful I. Kahn[1], Fahima Chowdhury[1], Nur Haq Alam[1], Edward T. Ryan[2], Stephen B. Calderwood[2], Firdausi Qadri[1]

[1]ICDDR,B, Dhaka, Bangladesh, [2]Massachusetts General Hospital, Boston, MA, United States

Vibrio cholerae O1 causes dehydrating diarrhea with a high mortality rate. However, the infection elicits long-term protective immunity against subsequent disease. Since *V. cholerae* is noninvasive, mucosal immunity is likely important for protection. In this study, we characterized cellular and humoral immune responses in the duodenal mucosa and in blood of patients with cholera in an endemic setting. Duodenal biopsies were obtained from eighteen adult Bangladeshi patients 2, 30 and 180 days after presentation with cholera. Five healthy adults evaluated in a study of asymptomatic *H. pylori* infection were used as a comparator group. Mucosal and systemic immune responses were assessed by ELISPOT, ELISA and flow cytometry. In the acute stage of cholera, we observed selective recruitment of CD3+CD4+ T-cells to the lamina propria, but no significant increase in the proportion of CD3+CD8+ or B-cells, suggesting CD4+ T-cells in particular may play an important role in the generation of subsequent adaptive immune responses. Systemic immune responses peaked early after infection and returned to baseline levels by six months after infection. Duodenal IgA antibodies directed against cholera antigens also peaked early after infection, on day 30, and returned to baseline levels by six months after infection. However, a significant increase in IgA antibody secreting cell (ASC) responses to lipopolysaccharide (LPS) in lamina propria lymphocytes (LPL) compared to healthy controls, was found on all study days ($P<0.05$ for days 2, 30 and 180). These mucosal ASC responses peaked on day 30, but were also evident in patients only 2 days after onset of illness, suggesting that in an endemic area, patients mount an anamnestic mucosal immune response to *V. cholerae* antigens. Although duodenal ASC responses waned by day 180, they remained significantly elevated compared to healthy controls, even when serum antibody responses had returned to control levels. These data suggest an early CD4 T-cell response in the gut mucosa may be associated with a subsequent influx of antigen-specific antibody secreting cells that may help mediate protection at the mucosal surface.

485

QUANTITATIVE REAL TIME PCR (QRT-PCR) FOR ENTHEROPATHOGENIC *E. COLI* (EPEC) IN STOOL SAMPLES FROM CHILDREN WITH AND WITHOUT DIARRHEA

Francesca Barletta[1], Erik Mercado[1], Theresa J. Ochoa[1], Lucie Ecker[2], Ana I. Gil[2], Claudio F. Lanata[2], Thomas G. Cleary[3]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Instituto de Investigación Nutricional, Lima, Peru, [3]University of Texas School of Public Health, Houston, TX, United States

EPEC is a main pathogen in children with diarrhea, however it can be identified also in a significant percentage of healthy controls. The aim of this study was to compare the bacterial load of EPEC in stool samples from children with and without diarrhea. We have analyzed 143 stool samples positive for EPEC detected by PCR from Mac Conkey plates; 53 from diarrhea cases and 90 from healthy controls in children under 2 years of age in Lima, Peru. DNA was isolated from stool specimens by a cetyltrimethylammonium bromide (CTAB) extraction method. Primers and probes were designed to amplify and quantify the intimin (*eaeA*) gene of EPEC2348/69 in a single reaction by qRT-PCR. To standardize the method, a direct correlation was determined between the fluorescence threshold cycle (CT) and the copy number of the *eaeA* gene. The standard curve was constructed by using known quantities of genomic DNA from 1.04 x 100 to 1.04 x 108 fg (1 molecule of DNA = 5.44fg of DNA genbank NC011601). A mixture of all PCR reagents without any DNA was used as a negative control. The detection limit of this PCR assay was 5 copies of the *eaeA* gene per mg of stool. The geometric mean of the bacterial load on the diarrhea group was 299.5 bact/mg (95%CI: 77.1-1,163.9) vs. 28.8 bact/mg (95%CI: 9.5-87.3) in the control group ($p=0.016$). Among children younger than 12 months of age the bacterial load in the diarrhea group was higher than the controls (177.6 vs 5.1 bact/mg, $p=0.006$); there were no significant differences among older children. Among children with EPEC as a single infection the bacterial load in the diarrhea group was higher than the controls (463.5vs 24.5 bact/mg, $p=0.006$); there were no significant differences among children with co-infections. In conclusion, the bacterial load of EPEC, measured by qRT-PCR on stool samples, is higher in children with diarrhea than in healthy controls. qRT-PCR is a potential useful tool to study the relation between disease and colonization by enteric pathogens.

486

IMMUNOGENICITY AND PROTECTIVE EFFICACY OF A FIMBRIAL ADHESIN-BASED VACCINE AGAINST ENTEROTOXIGENIC *ESCHERICHIA COLI* IN *AOTUS NANCYMAAE*: EVALUATION OF DOSING AND ROUTE OF VACCINATION

Ryan C. Maves[1], **Michael J. Gregory**[1], David Cepeda[1], Gladys Nuñez[1], Ana Cevallos[1], Nereyda Espinoza[1], Aisling O'Dowd[2], Stephen J. Savarino[2]

[1]United States Naval Medical Research Center Detachment, Lima, Peru, [2]United States Naval Medical Research Center, Silver Spring, MD, United States

Enterotoxigenic *Escherichia coli* (ETEC) is a major cause of morbidity and mortality worldwide. Previous studies have demonstrated the protective efficacy of a 4-dose intranasal (IN) regimen of CfaE, the tip adhesin of CFA/I, admixed with the B subunit of cholera toxin (CTB) to orogastric challenge with ETEC strain H10407 (CFA/I, O78:H11, LT+/ST+). In this study, we replace CTB with the B subunit of ETEC heat-labile toxin (LTB) and compare the 4-dose IN regimen to a 3-dose IN regimen and to a novel 3-dose intradermal (ID) route. 32 adult *Aotus* were assigned to 4 groups of 8 animals each. Group I received 4 vaccine doses by the IN route on days 0, 14, 28, and 84. Groups II and III received 3 vaccine doses by the IN and ID route, respectively, on days 0, 14, and 28. Group IV control animals received IN phosphate-buffered saline (PBS) on days 0, 14, 28,

and 84. Doses contained 200 µg dsc19CfaE and 290 µg rLTB (for IN) or 100 µg dsc19CfaE and 145 µg LTB (for ID) in PBS. 14 days after the final vaccine dose, all animals were orally challenged with 5x1011 CFU of ETEC strain H10407. Diarrhea attack rates were 12.5%, 37.5%, 50%, and 75% in groups I, II, III, and IV, respectively. The mean respective durations of diarrhea were 2, 6.7, 4.3, and 5.5 days. Significant protection was seen only in the 4-dose IN group when compared to controls (p=0.041). Serum IgG and IgA antibody titers to CfaE rose after the second or third dose by the IN route and after the first dose for in the ID group. In groups I and III, peak serum IgG and IgA titers were noted the day before challenge, whereas in group II titers peaked after challenge. Serum IgG responses to LTB rose after the second dose in all groups, peaking the day before challenge. In conclusion, an ETEC vaccine comprising CfaE and LTB, given in a 4-dose IN regimen, significantly protects *Aotus nancymaae* from diarrhea following homologous oral challenge with ETEC. The same vaccine given in a 3-dose IN or 3-dose ID regimen is immunogenic but did not significantly reduce the diarrheal attack rate compared to controls.

487

MORTALITY TRENDS OBSERVED IN POPULATION-BASED SURVEILLANCE OF AN URBAN INFORMAL SETTLEMENT, KIBERA, KENYA, 2007-2009

Beatrice Olack[1], Daniel R. Feikin[2], Leonard O. Cosmas[1], Kennedy O. Odero[1], George O. Okoth[1], Robert F. Breiman[2]

[1]Kenya Medical Research Institute/Centre for Disease Control and Prevention, Nairobi, Kenya, [2]International Emerging Infections Program-Kenya, United States Centers for Disease Control and Prevention, Nairobi, Kenya

Rapid urbanization in sub-Saharan Africa has led to new and expanded informal settlements, where dense populations with sub-standard hygiene and sanitation threaten health of residents. Mortality among residents of such settlements has not been well-characterized. In 2005, we established population-based infectious disease surveillance (PBIDS) within Kibera, the largest contiguous slum in Africa. Surveillance is conducted within two villages with nearly 30,000 participants. Field workers visit each household bi-weekly to identify all illnesses and deaths. Each participant has free access to a field clinic staffed by health care workers trained in PBIDS protocol. We analyzed data from January 2007 to December 2009 on participants who died during this period, focusing on illnesses and health seeking behavior reported in home visits. Person-years of observation (pyo) were based on weekly counts of participants residing within study area. We reported 566 deaths; overall mortality rate was 7.0 (95% CI 6.9-7.0) per 1,000 pyo. Mortality rate for children ≤5 years old was 15.2 (95% CI 15.1-15.2) per 1,000 pyo, 3-fold higher than that for persons >5 years old (5.1 per 1,000 pyo, 95% CI 5.0-5.1).Mortality rate for neonates was 95.3 (95% CI 93.7-96.9) per1000 pyo. Female infants had higher mortality rates than male infants (Rate ratio = 1.60; 95% CI 1.09 - 2.35). In contrast, for persons >5 years old, females had significantly lower mortality rates than males (RR= 0.77; 95% CI 0.62 - 0.95). Most children 74 (82%), ≤5 years old had at least 1 of the following: cough 34 (38%), diarrhea 41 (46 %), or fever 50 (56%); and persons >5 years old; 110(82%) had: cough 43(39%); diarrhea 28(26%) and fever 88(80%). Many of these symptoms overlapped. Accident and injuries accounted for 15(7%) total deaths that occurred within two weeks. Children ≤5 years old were two times more likely to receive clinic or hospital care than persons >5 years during the 2 week period before death. In conclusion, gender differences in mortality by age group require further study, but may reflect differential timing of health care access (for infants) and risk factors for severe disease (for older participants). A high proportion of deaths appear to be associated with infectious disease symptoms; disease prevention programs need to include focus on informal settlements, where residents have traditionally been neglected in health promotion efforts.

488

HEALTH EFFECTS AND COPING STRATEGIES TO FLOODS IN KUMI DISTRICT, EASTERN UGANDA

Peter Kirabira, Christopher G. Orach, David Mukanga

Makerere University College of Health Sciences, School of Public Health, Kampala, Uganda

Globally, floods account for over 40% of natural disasters and contribute to 37% of all mortality. During 2007, Uganda experienced prolonged floods that affected the eastern and northern districts including Kumi. We assessed the health effects and coping mechanisms to the 2007 floods in Kumi district.

Methods We conducted a cross-sectional study during May 2008. Data was collected from all the 26 health facilities in Kumi district using record reviews and 17 key informant interviews were conducted. Quantitative data were analyzed using SPSS version 13.0 while qualitative data were analyzed using Manifest-Content Analysis technique. The leading causes of morbidity were malaria (OPD:45.44%; IPD:53.13%), respiratory infections (OPD:14.14%; IPD:9.42%) and injuries (OPD:3.40%; IPD:3.71%), while malaria (27.36%), respiratory infections (9.92%) and injuries (4.62%) were the leading causes of mortality. Diarrheal diseases (4.42%), injuries (3.09%) and respiratory infections (1.57%) had highest case fatality rates. Under-fives were most affected (OR=1.06, 95%CI 1.01-1.11), females were more likely to be admitted during the floods than before or after (OR=1.05, 95%CI 1.00-1.10). There was higher under-five mortality during the floods (OR=1.72, 95%CI 1.22-2.69) and after (OR=1.76, 95%CI 1.15-2.61) compared to before. 15/26 health facilities had disruption of routine services; there was no rapid response team prior to the floods. Main challenges were lack of district disaster management plan, low budget, understaffing and sustainability of clean safe water. In conclusion, the main causes of morbidity and mortality were communicable diseases (malaria, respiratory infections) and injuries. Under-fives and women were most vulnerable. There is need for the district to develop a disaster management plan and budget, focusing on communicable disease control, injuries, and on vulnerable under fives and women during disaster response and recovery period. The district should set up early warning systems to improve disaster management.

489

SEROPREVALENCE OF ZOONOSES IN MONGOLIA: SURVEILLANCE AND RISK FACTOR ASSESSMENT

Roman Wölfel[1], Damdin Altantuul[2], Ilona Mossbrugger[1], Lhagvajav Zorig[2], Biraasuren Enkhtuvshin[3], Rendoo Davaadorj[4]

[1]Bundeswehr Institute of Microbiology, Munich, Germany, [2]Central Clinical Hospital of the Mongolian Armed Forces, Ulaanbaatar, Mongolia, [3]General Authority for Border Protection of Mongolia, Ulaanbaatar, Mongolia, [4]Mongolian Armed Forces General Staff, Ulaanbaatar, Mongolia

Public health surveillance in Mongolia currently relies, primarily, upon passive disease reporting from health care providers. It is suspected that there is presently a substantial underreporting especially of various zoonotic infections. The true prevalence of infections such as plague, tularaemia, brucellosis, tick-borne encephalitis (TBE), Q-fever, Congo-Crimean Hemorrhagic Fever (CCHF) and hanta virus infections within Mongolia is unknown. Therefore, we performed a seroprevalence study in five different regions of Mongolia in order to determine the prevalence of exposure to the disease agents of interest and to assess the demographics associated with prior exposure to these infections. During the study period blood samples were obtained from 765 consenting members of the Mongolian Armed Forces (670 male, 95 female). All participants were asked to complete a questionnaire identifying their demographics, medical history and possible risk factors for exposure to these infections. Samples were tested for disease-specific IgG antibodies by ELISA and/or immunofluorescence assays. Results indicate no or infrequent exposure to the CCHF virus (2.9%) and *Francisella tularensis* (1.7%). In contrast

to this, high seroprevalence rates for *Yersinia pestis* (22.6%), *Brucella sp*. (14.9%), *Coxiella burnetii* (13.9%) and hanta viruses (10%), suggest that human infections with these zoonotic bacteria are frequent and largely unrecognized in Mongolia. Exposure to *Y. pestis* (range 7.3-38.8%) and TBE virus (range 0.6-29.8%) appeared to be significantly different between certain rural regions of Mongolia. Demographic features of seropositive persons did suggest distinct epidemiology, ecology and risks for brucellosis, TBE and plague, whereas specific associations between the other diseases and certain risk factors could not be demonstrated. The results of this first nationwide study allow an estimation of the baseline disease prevalence for the above mentioned infections among the Armed Forces in different regions of Mongolia. Further investigations are necessary to develop a better understanding of risk factors important for the reduction of exposure of these zoonotic diseases in Mongolia.

490

TEN-YEAR CANCER TREND AND AVAILABLE INSTITUTIONAL CAPACITIES FOR RESPONSE IN UGANDA, 2010

Moses Tetui, Suzanne Kiwanuka, Danstan Bagenda

Makerere University College of Health Sciences, School of Public Health, Kampala, Uganda

Globally, cancer is the biggest non communicable disease (NCD); estimated to have accounted for about 13% of all deaths in 2007. The burden of NCDs for developing and newly industrialized countries is expected to rise over 60% by 2020 compared to a rise of 10% in developed countries. This study established the cancer trends (1999 to 2008) from the pathology department of Mulago National Referral Hospital, describes the demographic distribution and assessed institutional capacities for response in Uganda. This was a descriptive retrospective study, which employed quantitative and qualitative methods. Records were reviewed to establish cancer trends. We held key informant interviews with leaders at various departments that offer cancer related services to assess institutional capacities. Quantitative data was analysed using STATA Version 10 and qualitative data analysed using thematic manifest techniques. The most commonly diagnosed cancers between 1999 and 2008 were; Kaposi's sarcoma, cervical cancer and cancer of the eye. The three commonest cancers in men were Kaposi's sarcoma, prostate and cancer of the eye, while in women it was cancers of the cervix, breast and ovary. Majority of the cancers are on the increase in Uganda and females contribute to 58% of all the cancers diagnosed. The median age for cancer diagnosis was 40 years (IQR 27, 55). The proportion of persons affected by breast cancer has significantly increased between 1999 and 2008 (82 cases vs 142, $p<0.001$). HIV related cancers are still prevalent although they are declining. Critical service gaps identified were: inadequate access to diagnostic and treatment services and shortage of human resources. In conclusion, there is a steady increase of cancer in Uganda with females being more affected. The existing institutional capacities are insufficient to match the increasing trend. There is an urgent need for government and partners to increase human resources and accessibility to diagnostic and treatment services for cancers in order to improve cancer outcomes.

491

THE EFFECTS OF MATERNAL HELMINTH INFECTION AND CO-INFECTION WITH MALARIA ON BIRTHWEIGHT AND SUBSEQUENT GROWTH IN OFFSPRING IN A POPULATION ON THE COAST OF KENYA

Jessica K. Fairley[1], Indu Malhotra[1], Peter Mungai[2], Eric Muchiri[2], Christopher L. King[1], Charles H, King[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]Division of Vector Borne Diseases, Nairobi, Kenya

While there is abundant data on the effects of maternal malaria infection during pregnancy on the infant, data on the effects of maternal helminth infection and malaria-helminth co-infection on the offspring are lacking. We seek to better define the associations between maternal infection and early childhood growth in an endemic area. A cohort of pregnant woman and their infants from the Kwale district in Kenya were followed prospectively. The women were tested for malaria and helminth infection at delivery and birthweight was documented. Subsequent height, weight, hemoglobin, and malaria infection status were measured on the offspring every 6-12 months up to age 60 months. There were 696 live births of full-term infants and 2072 follow-up data points. 42.7% of the mothers were infected with *Plasmodium falciparum*, 30.6% with Schistosoma haematobium, 36.2% with filariasis, 31.5% with hookworm, and 5.9% with Trichuris trichiura. 29.5% were infected with *P. falciparum* and helminthes, 13.2% with *P. falciparum* alone, 41.1% with one or more helminthes, and 16.2% with none of the parasites. 8.3% of the infants had low birthweights with a z-score of -2 SD or below. There were no significant differences in mean birthweights between those with no infection and the other three infection groups. In the follow-up data, the percentage of underweight ranged from 13.6%-19.2%. For height, the data showed a range of prevalence of stunting of 40.2-50.7%. There were no significant differences in height z-scores on univariate analysis between the four infection groups described above, however, in most age groups, the "no infection" group tended to have a significantly worse mean weight z-score than the other groups. This data analysis does not show a significant effect of maternal infection on infant growth, however, the relatively small percentage of mothers without infection may make it insufficiently powered to detect a true difference. Multivariate analysis controlling for presence of malaria infection and anemia in the children may also prove to unmask differences.

492

A SYSTEMATIC REVIEW OF SAFETY DATA REPORTING FROM MALARIA, TUBERCULOSIS, AND HIV VACCINE TRIALS: THE NEED FOR INTERNATIONAL GUIDELINES

Cindy Tamminga[1], Santina Maioletesi[1], Charlotte Fedders[1], Jan Bonhoeffer[2], Uli Heininger[2], Vasee Moorthy[3], Thomas Richie[1], Judith Epstein[1]

[1]United States Military Malaria Vaccine Program, Naval Medical Research Center, Silver Spring, MD, United States, [2]Division of Infectious Diseases and Vaccines, University Children's Hospital, Basel, Switzerland, [3]Initiative for Vaccine Research, World Health Organization, Geneva, Switzerland

Malaria, tuberculosis and HIV combine to kill approximately 5 million people each year with the poor of the world most severely affected. The development and testing of promising candidate vaccines for each of these three poverty related diseases (PRD) is of critical importance. The speed and efficiency of developing safe and effective vaccines would be facilitated if researchers could objectively compare the clinical safety results of trials conducted in various settings. As a first step to creating a standardized reporting format, we assessed the current reporting practices of safety data from clinical trials of candidate vaccines for these PRD. A systematic literature review was performed of articles published in the English language during the time period January 2000 to June 2009. Approximately 150 articles met inclusion criteria and were reviewed. Differences in the methods of collecting, analyzing, and reporting adverse events were evaluated. Particular attention was paid to unique challenges related to conducting clinical trials in resource poor countries. The results of this review, demonstrating the heterologous nature of safety data reporting, will be presented. The Brighton Collaboration, an international voluntary collaboration to facilitate the development, evaluation, and dissemination of high quality information about the safety of human vaccines, will utilize the results of our extensive review to develop guidelines aimed at improving the detail, accuracy, completeness, and comparability of vaccine safety data from clinical trials conducted on vaccines for PRD.

493

COMPUTER-BASED MODELING IN SUPPORT OF GLOBAL ERADICATION OF INFECTIOUS DISEASES

Phillip Eckhoff

Intellectual Ventures, Bellevue, WA, United States

Malaria and polio are current targets of Global Eradication campaigns, and success of these campaigns and any future disease eradication campaigns will provide lasting benefits to humanity. Modern computing and modeling can assist rational planning of disease eradication campaigns to maximize the probability of success in the face of significant challenges and obstacles. We present a new computational framework which is designed to answer questions posed by Eradication campaigns with specific focus on malaria as an example. New detailed models for single malaria infections and mosquito population dynamics are developed and integrated into a large spatial-scale dynamic simulation. The single malaria infection model includes detailed descriptions of parasite intrahost development and human immunology which combine to provide mechanistic explanations of phenomena such as infection duration and adapted response to re-infection. The model for mosquito population dynamics captures the effects of multiple simultaneous vector control interventions upon the mosquito population and the resulting change in parasite population dynamics. The integration of these detailed micro-models into a large-scale spatial simulation with individual resolution allows study of many possible combined-intervention malaria eradication campaigns. Overall probability of campaign success for different combined approaches in the presence of systemic, campaign, and model uncertainty is studied and conclusions for locally-tailored approaches are discussed.

494

ENVIRONMENTAL PRESSURE ON THE ANTIBODY RESPONSE TO A CHILDHOOD VACCINE IN NORTHERN SENEGAL

Lobna Gaayeb Juliàn[1], Emmanuel Hermann[2], Jean-Baptiste Hanon[3], Jean Biram Sarr[1], Mamadou Ousmane Ndiath[4], Anne-Marie Schacht[1], Franck Remoué[5], Gilles Riveau[3]

[1]Laboratoires de Recherche Médicale - Espoir Pour la Santé, Saint-Louis, Senegal, [2]Université Lille Nord de France, Lille, France, [3]Centre of Infection and Immunity of Lille - Unité 1019 Institut National de la Santé et de la Recherche Médicale, Lille, France, [4]Unité Mixte de Recherche 198 - Institut de Recherche pour le Développement, Dakar, Senegal, [5]Unité de Recherche 016 - Institut pour la Recherche et le Développement, Cotonou, Benin

Environmental factors play a role in vaccine induced immunity. Season-dependent elements have particularly been involved in the modulation of immune responses in developing countries. In Senegal, a sub-Saharan country with two distinct seasons, a dry and a wet one, we conducted a study to investigate whether there is a modulation of the immune response to a childhood vaccine according to seasonal factors. Whooping cough is a vaccine-preventable respiratory disease caused by *Bordetella pertussis* infection, against which Senegalese children are immunized with the Diphteria-Tetanus-whole Pertussis vaccine (DTwP). To assess the level of immunization against whooping cough, we conducted a cross sectional and longitudinal study (1.5 year) in which serum samples were collected from 410 children aged 1 to 10 from 5 villages in Northern Senegal. We tested these sera for antibodies (Ab) against two major antigens of *B. pertussis*: filamentous hemagglutinin (FHA) and pertussis toxin (PT). Although most children were immunized with DTwP, FHA-specific IgG response was significantly different according to age. Until the age of 5, response to FHA was low, and got higher in the older group. Assessment of anti-PT IgG response suggested evidence of recent exposures to the pathogen. Moreover, IgGs to another antigen included in the DTwP vaccine, the tetanus toxoid (TT), was quantified. A high specific Ab response, which decreased with age, was observed. This suggests that the detected low levels of FHA-specific Ab, especially in the younger group of children, were not due to a failure in vaccination. Noteworthily, significant differences in the specific Ab responses to FHA, PT and TT were observed between villages in the same studied area. Besides, when the results from sera collected every three months were compared, a strong effect of seasonal factors on the Ab response to DTwP antigens was detected. The results of this work should be critical in the scope of a better understanding of the role of environmental factors on the establishment and maintenance of immunity to vaccines.

495

MALARIA INCIDENCE AND PREVALENCE AMONG CHILDREN LIVING IN A PERI-URBAN AREA ON THE COAST OF BENIN, WEST AFRICA: A LONGITUDINAL STUDY

Alain M. Nahum[1], Annette Erhart[2], Ambroisine Mayé[3], Daniel Ahounou[1], Chantal van Overmeir[2], Joris Menten[2], Harry van Loen[2], Harry van Loen[2], Martin Akogbeto[1], Marc Coosemans[2], Achille Massougbodji[4], Umberto D'Alessandro[2]

[1]Centre de Recherche Entomologique de Cotonou, Cotonou, Benin, [2]Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium, [3]Hôpital de Zone, Abomey-Calavi, Benin, [4]Laboratoire de Parasitologie, Faculté des Sciences de la Santé, Cotonou, Benin

Clinical malaria incidence was determined over 18 months in a cohort of 553 children living in a peri-urban area near Cotonou. Three cross-sectional surveys were also carried out. Malaria incidence showed a marked seasonal distribution with 2 peaks, the first corresponding to the long rainy season and the second to the overflowing of Lake Nokoue. The overall *Plasmodium falciparum* incidence rate was estimated at 84/1,000 person-months, its prevalence at over 40% in the two first surveys and 68.9% in the third. Multivariate analysis showed that girls and people living in closed houses had a lower risk of clinical malaria. Bed net use was associated with a lower risk of malaria infection. Conversely, children of families owing a pirogue were at higher risk of clinical malaria. Considering the high pyrethroids resistance, indoor residual spraying with either a carbamate or an organophospate insecticide may have a major impact on the malaria burden.

496

COMBINED UTILITY OF TOURNIQUET TEST AND WHITE BLOOD CELL COUNT AS TRIAGE CRITERIA FOR DENGUE IN THE AMERICAS

Christopher Gregory[1], Luisa Alvarado-Domenech[2], Lissy Colon[3], Ramon Cruz-Rivera[3], Liv Cuyar-Bermudez[3], Ivonne Galarza[2], Carlos Garcia-Gubern[3], Olga Lorenzi[1], Fernando Ortiz-Baez[3], Luis Santiago[1], Kay Tomashek[1]

[1]Centers for Disease Control and Prevention, San Juan, Puerto Rico, [2]Department of Pediatrics, San Lucas Hospital/Ponce School of Medicine, Ponce, Puerto Rico, [3]Department of Emergency Medicine, San Lucas Hospital/Ponce School of Medicine, Ponce, Puerto Rico

As the clinical presentation of dengue can be non-specific, and rapid diagnostic tests are not readily available, finding easily obtainable markers that can distinguish dengue from other acute febrile illnesses is a priority. Data from Thailand suggests that the combination of a positive tourniquet test (TT) and leukopenia can distinguish dengue from other febrile illnesses in children; little data exists on the utility of these tests in adults or in the Americas. We evaluated the utility of the TT and leukopenia (white blood cell count <4000/mm^3) for identifying dengue as part of a febrile illness surveillance study conducted in the Emergency Department of the Hospital San Lucas in Ponce, Puerto Rico. From September to December 2009, 284 patients presenting to the ED with fever for 2-7 days and no identified source of infection were enrolled. Participants were tested for influenza, dengue, leptospirosis, and enteroviruses. Thirty-one (10.9%) of patients were confirmed as having dengue; a definitive etiology was determined for 142 others (136 influenza, 2 leptospirosis, 3 enterovirus) and 111 patients had no infectious etiology identified. Fifty-two percent of laboratory-positive dengue cases had a positive TT versus 18% of

patients without dengue (p <.0001), and 71% of dengue cases compared to 22% of non-dengue cases had leukopenia (p<0.001). The combination of a positive TT and leukopenia had a sensitivity of 39%, specificity of 96%, positive predictive value (PPV) of 52% and negative predictive value (NPV) of 93%. Having either a positive TT or leucopenia was 84% sensitive, 71% specific, and had a PPV of 27% and NPV of 97%. The tourniquet test was more sensitive in dengue patients with a platelet count of >100,000 than in patients with a count <100,000, but this was not significant (78% vs. 41%, p=0.11). Our study supports the combined use of the tourniquet test and leukopenia as useful markers of dengue infection in adults and children in Puerto Rico. Few patients with early dengue infection would be missed using these tests as triage criteria.

497

COMMUNITY-BASED STUDY OF CHAGAS DISEASE PREVALENCE IN LOS ANGELES COUNTY

Mahmoud I. Traina[1], Salvador Hernandez[1], Louis V. Kirchhoff[2], Aiman M. Smer[1], Haneen Khamag[1], Eva Padilla Garcia[1], Sheba K. Meymandi[1]

[1]Olive View-University of California at Los Angeles Medical Center, Sylmar, CA, United States, [2]University of Iowa, Iowa City, IA, United States

Chagas disease (CD), caused by the protozoan *Trypanosoma cruzi*, causes the most important parasitic disease burden in Latin America, where an estimated 8 million persons are infected. Chronic CD results in symptomatic cardiac and/or gastrointestinal disease in 10-30% of infected persons, and each year roughly 20,000 deaths are attributed to the illness in the endemic countries. Approximately 17 million persons born in the countries in which CD is endemic currently reside in the U.S. and roughly 300,000 of these immigrants are thought to have chronic CD. Considerable information regarding CD prevalence among U.S. blood donors has been published, but community-based perspectives on CD prevalence are largely lacking. The Chagas Community Screening Project was initiated in Los Angeles County in April 2008 by the Center of Excellence for Chagas Disease at Olive View-UCLA Medical Center. Randomly-selected adults 18 to 60 years old residing in Los Angeles County who had lived in a Chagas-endemic country for 1 year or more were enrolled in the study, mostly through church groups. Blood samples were screened serologically in a prototype ELISA in which chimeric recombinant proteins FP3, FP6, and FP10 were used as target antigens. Samples positive in the ELISA then underwent confirmatory testing in the Chagas RIPA. To date 985 subjects have been tested. The median age was 45. The endemic countries in which the subjects had spent 1 year or more were as follows: Mexico 719 (72.8%), El Salvador 141 (14.3%), Guatemala 77 (7.8%), Peru 17 (1.7%), Columbia 11 (1.1%), Honduras 7 (0.7%), Nicaragua 6 (0.6%), Costa Rica 3 (0.3%), Ecuador 2 (0.2%), Argentina 1 (0.1%), and Venezuela 1 (0.1%). A total of 10 subjects were RIPA-positive (1.0%). The median age of the positive subjects was 48. 5 patients were from Mexico (0.7%), 4 from El Salvador (2.8%), 1 from Honduras (14.3%), and none from the other countries. Our study demonstrates a substantial prevalence of CD in Latin American immigrants in Los Angeles County and suggests that similar numbers of persons with CD are present in other communities in which immigrants from Chagas-endemic countries have settled. Serologic screening of immigrants at geographic risk for CD should be performed so that appropriate monitoring and treatment can be carried out.

498

KNOWLEDGE, ATTITUDES, AND PRACTICES OF PRACTITIONERS WHO PROVIDE PRE-TRAVEL CONSULTATIONS

Laura Kogelman[1], Elizabeth D. Barnett[2], Emad Yanni[3], Michael R. Winter[4], Christine E. Chaisson[4], Lin H. Chen[5], Mary E. Wilson[6], Adolf W. Karchmer[7], Winnie W. Ooi[8], Erika Gleva[5], Gary Brunette[3], Davidson H. Hamer[9]

[1]Tufts Medical Center, Boston, MA, United States, [2]Boston Medical Center, Boston, MA, United States, [3]Division of Global Migration and Quarantine-Traveler's Health Branch, Centers for Disease Control and Prevention, Atlanta, GA, United States, [4]Data Coordinating Center, Boston University School of Public Health, Boston, MA, United States,[5]Mt. Auburn Hospital, Cambridge, MA, United States, [6]Harvard School of Public Health, Boston, MA, United States, [7]Beth Israel Deaconess Medical Center, Boston, MA, United States, [8]Lahey Medical Center, Burlington, MA, United States, [9]Center for Global Health and Development, Boston, MA, United States

International travel by US residents is increasing. Many health professionals are asked to provide pre-travel advice but may not be prepared to provide these specialized services. We designed an anonymous internet survey to assess knowledge, attitudes and practices of primary-care providers (PCP) and specialists who provide pre-travel consultations. The survey was sent to ~20,000 randomly selected PCPs in the Pri-Med Institute (now pmiCME) database and >3,000 US-based travel medicine specialists, identified from ISTM, ASTMH, and CDC yellow fever vaccine provider mailing lists. Of 14,932 e-mails sent to valid e-mail addresses, 902 yielded complete or partially completed surveys (6.0%). Respondents included 51% MDs, 25% NPs, 9.6% PAs, 8.5% RNs and 5.1% DOs. Identified specialties included Internal Medicine (30%), 32% Family Practice, 18% Primary Care, 9% Pediatrics, and 28% other. 87% were interested in attending a travel medicine course and 80% personally provided pre-travel consultations. 47% of the 625 respondents who provided pre-travel consultation saw fewer than 50 travelers/year, 15% saw 51-100, 26% 101-500, and only 12% >500/y. Familiarity with travel-specific vaccines (e.g. yellow fever, Japanese encephalitis) and provision of written educational materials increased as annual volume of travelers increased. More respondents who saw <30 travelers/y than those who see >500/y would prescribe chloroquine for malaria chemoprophylaxis for travel to sub-Saharan Africa (an inappropriate drug for this destination) (11% vs. 5.2%, p = 0.14). Those who saw <30/y were less likely to prescribe chloroquine (first-line drug) to travelers to malaria-endemic areas of Central America (36% vs. 81% who saw >500/y, p <0.001). When asked about *Campylobacter* antibiotic resistance in SE Asia, 32% of providers who saw <30/y incorrectly selected azithromycin resistance as a problem and 10% incorrectly thought antibiotic resistance was not an issue. Fewer providers who saw <30/y knew fluoroquinolone resistance was a problem (47% vs. 75% who saw >500/y, p <0.001). Many survey respondents provided pre-travel advice, but most saw very few travelers. Travel medicine knowledge and use of supplementary educational materials increased with volume of patients seen. Specific knowledge and practice deficits among study participants demonstrated a need for additional travel medicine education especially for those seeing fewer travelers.

499

CORRELATION OF BRAIN MR IMAGING AND CLINICAL MANIFESTATIONS OF EOSINOPHILIC MENINGITIS CAUSED BY *ANGIOSTRONGYLUS CANTONENSIS* IN SOUTHERN TAIWAN

Hung-Chin Tsai, Yu-Ting Tseng, Susan Shin-Jung Lee, Yao-Shen Chen

Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan

The most common cause of eosinophilic meningitis is the rat lungworm *Angiostrongylus cantonensis*, a parasite that is endemic in the southeast Asia and Pacific regions. Correlation of Brain Magnetic Resonance (MR)

imaging findings and clinical features are rarely reported in the literature. This study is aimed to analysis the Brain imaging features and clinical manifestations in patients with eosinophilic meningitis in southern Taiwan. This retrospective cohort study consisted of all of the patients diagnosed with eosinophilic meningitis at Kaohsiung Veterans General Hospital from December 1991 through Septemper 2009. The associations between laboratory parameters and brain MR imaging findings were analyzed by Mann-Whitney U test. Thirty-seven patients were diagnosed to have eosinophilic meningitis during a period of 18 years. Age ranged from 2 to 80 years. Most of the patients (35/37, 95%) were adults. The median incubation period was 10.5 days (range 3-80). Thirty percent of the patients complained of hyperesthesia. Patients who had hyperesthesia tended to have longer incubation period, low serum IgE levels and longer duration between onset of symptoms and spinal taps. Three patients presented with lymphocytic meningitis initially. Brain MR imaging was performed in 26 patients. Leptomeningeal enhancement was noted in 17 patients. Increased signal intensity at the subcortical white matter of bilateral cerebral or cerebellar hemisphere on T2-weighted and fluid-attenuated inversion recovery (FLAIR) images was seen in 10 patients. Those patients who had leptomeningeal enhancement and increased signal intensity on T2-weighted and FLAIR imagings tended to have younger age and short incubation period. (Mann-Whitney U test, $p<0.05$). The presence of brain MR imaging abnormalities were not associated with timing between onset of symptoms and spinal tapping. In conclusion, hyperesthesia, rarely found in patients with bacterial and aseptic meningitis, is relative common in our patients. Patients who had younger age and short incubation period were more likely had leptomeningeal enhancement and increased signal intensity in Brain MR imagings. The possibility of eosinophilic meningitis can not be totally excluded despite absence of eosinophilia initially in the CSF. Detailed food intake history and laboratory tests are important to obtain the correct diagnosis.

500

TREATMENT OF STUBBORN SCALP, SKIN AND NAILS INFECTION OF FUNGAL AND BACTERIAL ORIGINS

Praise I. Oronsaye

University of Benin, Benin City, Nigeria

The purpose of this study, was to determine the efficacy of this new lotion in treating fungal and bacterial infection of scalp, skin and nails infection that have defiled remedies by known and commonly prescribed anti-fungal and anti-bacterial drugs by Physicians. These infections are common health problems of people in tropical and developing countries of the world. Two hundred and fifty patients with various scalp, skin and nails infections of fungi and bacterial organisms attending University of Benin Teaching hospital from January to December 2008 were randomly recruited into the study. They comprised of 150 females and 100 males whose ages range from children, adult and the elderly. The infections includes eczema, ringworm, scabies, witlow, sores and bruises. The composition of the lotion is as follows: salicylic 20% wt/vol, absolute ethanol 100 mls, glycerin 3% vol/vol. The salicylic was dissolved in the ethanol and glycerin was added, mixed thoroughly and allowed to stand for not less than 3 hours before application. Application was made using cotton wool bud on the affected sites only. Satisfactory clinical response was achieved within 3-7 days depending on severity of the infection. We present in this study, the dramatic effect of this lotion 'magic bullet' in treating scalp, skin and nail infection of fungal and bacteria origins.

501

DETERMINANTS OF ANTIBIOTICS PRESCRIPTION FOR SCHOOLCHILDREN AT ALLADA, SOUTH BENIN

Ghislain K. Koura[1], André Garcia, T. Beheton[1], Philippe Deloron[1], Michel Cot[2], Jean-François Faucher[3]

[1]Mère et enfant face aux infections tropicales, Paris, France, [2]Faculté de Pharmacie, Université Paris Descartes, Paris, France, [3]Hôpital Saint-Jacques, Service des maladies infectieuses et tropicales, Besançon, France

The objective of this study was to study the determinants of antibiotics prescriptions for schoolchildren by nurses. Data were collected during a prospective study on treatment of parasitologically-confirmed cases of malaria in four schools (Allomé, Centre, Dankoli, Dogoudo) of the district of Allada in the Republic of Benin. One thousand six hundred thirty children were included from February till June 2008. For each patient, sociodemographic characteristics, reasons for consultation, final diagnosis and therapeutic prescriptions were collected. A malaria rapid diagnosis test was used to screen for malaria diagnosis. Data were entered and validated with Epidata® software, and analyzed with STATA 10® software. Fever was the first reason for consultation (57 %), followed by digestive (27%) and respiratory (24%) symptoms and skin lesions (17%). A malaria diagnosis was confirmed in 61% of the children attending for fever. Antibiotic was prescribed for 40% of children (21% with confirmed malaria diagnosis and 57% with a non-malarial-fever). We found a significant association between an antibiotic prescription and a respiratory infection diagnosis (OR [IC 95 %]: 41.09 [24.34-69.33]), and to a lesser extent between an antibiotic prescription and a cutaneous infection diagnosis (OR [IC 95 %]: 5.78 [4.20-7.97]). In conclusion, the rational use of the antibiotics is a major challenge in poor resource countries. A better knowledge of the determinants of antibiotics prescription is critical in order to establish rules of this rational use of antibiotics. We found that, by far, the diagnosis of respiratory infection is the main factor associated with an antibiotic prescription. Was this finding firmly established, further clinical research studies would be needed in order to find the most appropriate ways of restricting antibiotics prescriptions for children who complain with respiratory symptoms.

502

IS THE *IN SITU* INFLAMMATORY PROFILE CORRELATED WITH THE CLINICAL PRESENTATION OF HUMAN SPOROTRICHOSIS?

Fernanda N. Morgado[1], Armando O. Schubach[2], Monica B. Barros[2], Marta A. Santiago[3], Marcela Costa-Santos[1], **Fatima Conceiçao-Silva**[1]

[1]Oswaldo Cruz Institute - Fiocruz, Rio de Janeiro, Brazil, [2]Evandro Chagas Institute - Fiocruz, Rio de Janeiro, Brazil, [3]BioManguinhos - Fiocruz, Rio de Janeiro, Brazil

Sporotrichosis, caused by the fungus *Spotothrix schenckii,* is a granulomatous skin disease whose most common clinical presentations are the lymphocutaneous (LC) and fixed cutaneous (F) forms. Although these forms are clinically well characterized, little is known about the immunopathological processes that determine the difference between clinical presentations. The study was developed in order to evaluate the composition of the in situ inflammatory reaction and to correlate it with clinical aspects. The composition of the *in situ* inflammatory process was analyzed by immunohistochemistry and clinical data were collected from two groups of patients with sporotrichosis: LC (n = 19) and F (n = 11). LC patients presented a larger number of lesions ($p = 0.001$), longer disease duration ($p = 0.026$) and longer duration of treatment ($p = 0.049$) when compared to F group. A greater fungal burden was also observed in LC lesions (LC: 0 to 6.5 and F: 0 to 1.5; $p = 0.021$). The percentage of neutrophils was significantly higher in LC lesions (median: 24.7%) than in F lesions (median: 6.7%) ($p = 0.002$), as well as the percentage of CD4+ cells (LC median: 40.9% and F median: 30.0%, $p = 0.001$) and CD22+ cells (LC median: 15.3% and F median: 2.9%, $p = 0.048$), and the intensity of NOS2 expression ($p = 0.009$). In conclusion, LC patients

presented more lesions, that were clinically more severe and presented a longer duration. The more marked inflammatory character of LC lesions was related to the larger number of neutrophils and CD4+ cells and to higher NOS2 expression, as well as the larger fungal load. The host-mediated immune response in sporotrichosis shows some peculiar characteristics of cellularity and inflammatory activity that might be determinant for progression of the different clinical forms.

503

BIBLIOMETRIC REVIEW FOR MALARIA IN PREGNANCY

Anna M. van Eijk, Jenny Hill, Sue Povall, Alison Reynolds, Helen Woods, Feiko Ter Kuile

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

The Malaria in Pregnancy (MiP) Library (http://www.update-software.com/Publications/Malaria/) is a bibliographic database of literature relating to malaria in pregnancy. The MiP Library was created in 2005 and is a product of the MiP Consortium. We conducted a bibliometric review of published and unpublished reports to obtain a better understanding of the available number and sources of information for malaria in pregnancy and changes over time. Every four months, the literature is screened online for new information using a standardized search protocol. The published literature includes journal articles, books, reports, academic theses, and policy guidelines. The unpublished literature includes registered studies (including trials), unpublished theses, meeting reports and other unconventional literature. There is no language restriction. By May 2009, the MiP Library contained 4373 entries, consisting of 3317 journal articles (75.9%), 348 reports (8.0%), 135 academic theses (3.1%), 97 books or book chapters (2.2%), and 355 conference proceedings (8.1%), 74 registered studies (1.7%) and 47 'other' (0.9%). Most of the sources were in English (87.2%), followed by French (7.2%) and Spanish (1.7%). About a third of the source material was publicly available on the internet (36.4%), and the remaining accessible with restricted access (37.5%) or not available (26.1%). The number of journal articles increased from 40 publications in the 1960s, to 699 in the 1990s, and to 1932 between 2000 and 2009; articles were sourced from 884 different journals. Among the journal articles published since 1959, the top 3 sources were the Am J Trop Med Hyg (194), Trans R Soc Trop Med Hyg (132), and the Malaria Journal (104), followed closely by J infect Dis (93), Lancet (74), and Trop Med Int Health (68). In conclusion, the last decade has seen a dramatic increase in publications related to malaria in pregnancy; an increasing proportion is now publically available through online sources. The MiP Library is an excellent scholarly source for literature and systematic reviews related to malaria in pregnancy.

504

LABORATORY SURVEILLANCE FOR THE POSSIBLE INFECTIOUS CAUSES OF UNDIFFERENTIATED FEBRILE ILLNESSES IN THE COUNTRY OF GEORGIA

Tinatin Kuchuloria[1], Danielle V. Clark[2], Tamar Akhvlediani[1], Alexander Nanuashvili[3], Manana Makhviladze[4], Marine Kanashvili[4], Tengiz Tsertsvadze[5], Marina Endeladze[5], Maiko Chokheli[6], Tamar Chikviladze[6], Marine Chubinidze[6], Brent House[7], Margaret Farrell[7], Mohamed Abdel Maksoud[7], Matthew J. Hepburn[8], Guillermo Pimentel[7]

[1]I. Javakhishvili Tbilisi State University; Technology Management Company, Tbilisi, Georgia, [2]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [3]Service of Antimicrobial Chemotherapy, Tbilisi, Georgia, [4]V. Bochorishvili Anti-Sepsis Center, Tbilisi, Georgia, [5]Infectious Diseases, AIDS and Clinical Immunology Research Center, Tbilisi, Georgia, [6]National Center for Disease Control and Public Health, Tbilisi, Georgia, [7]Global Disease Detection and Response Program, United States Naval Medical Research Unit No. 3, Cairo, Egypt, [8]United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, United States

Information on the relevant infectious causes of undifferentiated febrile illness (UFI) in a region is essential for effective treatment and prevention. Presumptive treatment with antibiotics is common in the Caucasus, where laboratory diagnostics are not systematically used for zoonotic and vector-borne infections. Laboratory based UFI surveillance was conducted at 5 hospitals in Georgia, starting in May, 2008. Patients ≥4 years of age with fever ≥38°C for ≥48 hours were considered for inclusion. Blood culture and serologic testing (ELISA) were conducted for leptospirosis, brucellosis, West Nile virus (WNV) infection, Crimean-Congo hemorrhagic fever (CCHF), Q fever, tick-borne encephalitis (TBE), hantavirus infection, typhoid fever, and rickettsial infections. To date, 309 subjects have been enrolled in the study. Enrolled subjects represent all but 2 regions of Georgia. Fever of unknown origin (FUO) was the preliminary diagnosis in 86% of patients. The median duration of fever was 15 days, with the maximum duration of 1000 days; gradual onset of fever was noted on 75% of the cases. The majority of patients reported antibiotic use prior to enrollment (71%) and 40% of patients reported self-medicating. Several *Streptococcus* and *Staphylococcus* species were isolated. Samples were positive by serology for brucellosis (7%), hantavirus infection (6.8%), leptospirosis (5.5%), Q fever (4.5%), typhus group rickettsial infection (4%), and TBE (1.2%). Currently 177 samples have been tested with CCHF and WNV IgM ELISA; only 1 CCHF-positive sample was recorded. In conclusion, clinical awareness and laboratory capacity are essential to diagnose infectious etiologies of febrile illnesses. As a result of this study, physicians and public health authorities will be informed of the relative frequency of the studied pathogens. Information from this ongoing study will be utilized to enhance clinical suspicion and focus efforts to develop diagnostic capacity and treatment options for these infections in the Caucasus.

505

FACS ANALYSIS OF KEY INNATE IMMUNE CELLS IN PERIPHERAL BLOOD OF DENGUE PATIENTS

Jih-Jin Tsai[1], Yen-Hua Jen[1], Jung-San Chang[1], Hui-Mien Hsiao[2], Guey Chuen Perng[2]

[1]Tropical Medicine Center, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan, [2]Department of Pathology and Laboratory Medicine, Emory Vaccine Center, Emory University School of Medicine, Atlanta, GA, United States

The importance of innate immune system in defense of host against pathogens has been well documented. As the first line of host defense system, timing becomes a critical step in order to have an efficient effect on the engagement with and controlling pathogens. Dengue is a timing acute disease and frequently dengue patients do not see for helps until 2-3 days of prodrome occurs. Thus, the role of innate immune system in dengue patients remains largely unexplored. Comprehensive FACS

profiling of key innate immune cells in peripheral blood of dengue patients varying in onset of fever was performed. Four FACS panels were used to evaluate NK cells, platelet-leukocyte aggregates, inflammatory monocytes, and plasmacytoid and myeloid dendritic cells, respectively. Serological results revealed that majority of dengue patients were primary dengue. FACS results showed the followings: i)NK cells, CD56+CD16+ or CD56+CD16-, were significantly dropped on the 5th day after onset of fever and were gradually resumed to normal within two weeks of illness. ii)Biphasic platelet-leukocyte aggregates was observed; reached to maximum levels on the 6th-8th days and on the 11th-16th days after onset of fever. The platelet-monocyte aggregates were the most frequent event. iii)Inflammatory monocytes, CD14+CD16+HLADR+, were consistent lower on 5th-8th days after onset of fever, and were gradually returned to normal level in the second week of illness. iv)Plasmacytoid dendritic cells, CD3-CD20-CD14-CD123+CD11c-, reached to the maximum on the 5th day after onset of fever, and were gradually declined to the baseline level after one week of illness. In contrast, myeloid dendritic cells, CD3-CD20-CD14-CD11c+CD123-, were somewhat fluctuated during the first week of illness, and thereafter returned to baseline level in the second week of illness. These results were the first phenotypically documented the key innate immune elements in peripheral blood of dengue patients. The most interest findings in current investigation was the biphasic platelet-leukocyte aggregates, in particular the platelet-monocyte aggregates. Perhaps these innate immunological parameters may be a crucial factor, which could dictate in understanding the complicated pathogenesis of dengue disease.

506

PREVALENCE OF ROTAVIRUS AMONG PATIENTS WITH ACUTE DIARRHEA IN DIFFERENT REGIONS OF UZBEKISTAN

Gulnara Ibadova[1], Adkham Mamatkulov[2], Gulnara Abdukhalilova[3], Ruslan Madyarov[3], Aybek Khodiev[2], Carl Mason[4], Ladaporn Bidhidatta[4]

[1]Tashkent Institute of Postgraduate Medical Education, Tashkent, Uzbekistan, [2]Technology Management Company, Tashkent, Uzbekistan, [3]Research Institute of Epidemiology, Microbiology and Infectious Diseases, Tashkent, Uzbekistan, [4]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

The rotavirus is one of the major causes of diarrhea. The purpose of study was to reveal prevalence and pattern of rotavirus diarrhea in different regions of Uzbekistan. 2450 stool samples from patients with acute diarrhea (AD) were collected from different regions of Uzbekistan. All samples were tested for bacterial diarrheagenic pathogens with culture on standard selective media and biochemical tests followed by serotyping. The rotavirus was detected with commercial ELISA following the instruction of commercial kit (IDEIA, DakoCytomation, UK). Rotavirus identified in 40.04% of samples (981 out of 2450 samples). The prevalence of rotavirus detection differed by regions. The high prevalence of rotavirus reveled in south regions of Uzbekistan (Kashkadarya and Surkhandarya regions and Tashkent city) - 43.1%, 44.69% and 43.13% accordingly. In Karakalpak republic and Khorezm region rotavirus identified in 37.88 and 33.71% accordingly. The difference of rotavirus identification revealed between inpatient and outpatient hospitals - 39.0% and 46.0% accordingly. Rotavirus was only identified pathogen in 25.35% of patients, the association of rotavirus and other diarrheagenic pathogens identified in 14.61% of patients. The most frequent association of rotavirus identified with the following pathogens (out of all associations): E. coli - 68 samples (19.0%), Citrobacter spp. - 65 samples (18.16%), Enterobacter spp. - 62 samples (17.32%), Salmonella typhimurim - 43 samples (12.01%) and Shigella spp. - 32 samples (8.94%). The significant seasonal prevalence of rotavirus diarrhea was not observed. In conclusion, 1) Rotavirus may be the cause of diarrhea in 40.0% of patients admitted with AD and has no seasonal prevalence; 2) rotavirus prevails in south regions of Uzbekistan; 3) rotavirus diarrhea more frequently found in outpatient oral rehydration facilities then in inpatients departments; 4) rotavirus as the only pathogen identified in 25.0% of AD cases and in 15.0% in association with other diarrheagenic pathogens.

507

EVALUATION OF FEVER IN AN INTERNATIONAL TRAVELER

Adetunji Adejumo[1], Abhijeet Nadkarni[2], Adenike Adeyinka[1], Shefiu Shittu[1], Olutayo Olabige[1], Cyrus Badshah[1]

[1]Harlem Hospital/Columbia University, New York, NY, United States, [2]Springs Memorial Hospital, Lancaster, SC, United States

The differential diagnosis of fever in an international traveler is broad and often times presents a diagnosis dilema for clinicians. Travel medicine has become an important part of medical practice in a world that is now a global village. The patient was a 57yrs old Caucasian man who returned 5days prior to presentation from a 1 week trip to New London, South Africa. He complained of fever-102ºF, severe headache, fatigue, lower extremity itching with erythematous rash and right groin swelling that started 1day before he left South Africa. He reported camping activity abroad and noticed ticks on his body. He denied mosquito bites or animal contacts and no sexual exposure. He had no diarhoea or dyusria. He had no improvement after course of keflex used upon arrival. On examination, he was febrile to 102ºF and stable. The right lower extremity skin had multiple erythematous patches measuring 1-1.5cm in diameter on the medial aspect of popliteal surface resembling eschars. There was right inguinal lymphadenopathy and no penile ulceration, discharge or scrotal swelling. Routine laboratory studies were normal. Based on the history and physical findings, a working diagnosis of African tick bite fever by *Rickettsia africae* was made. Rickettsia serology was sent to an outside laboratory for confirmation. He was commenced on doxycycline 100mg bid. At 1 week follow up, his fever has resolved and the lower extremity eschar was clearing. He completed 2weeks of therapy with complete resolution of the skin lesions and symptoms. An important emerging infectious disease, the incidence of Rickettsia infections worldwide is estimated at 5.6-11% in groups of travelers returning from Sub-Sahara Africa who developed acute febrile illness after returning from Africa. The causative agent of African tick-bite fever, is transmitted by *Amblyomma hebraeum* and *A. variegatum* ticks. These ticks are common in western, central, and southern Africa. Adults rarely feed on humans, although nymphs attach more. In conclusion, a high index of suspicion for African tick-bite fever is needed in persons who seek treatment with a history of tick bites and clinical signs of fever, headache, and multiple eschars after traveling to an endemic area.

508

ANTIBODIES IN ACTION: ROLE OF OPSONINS IN CLEARING *SALMONELLA* TYPHI IN HUMANS

Janet C. Lindow, Kelly A. Fimlaid, Janice Y. Bunn, Beth D. Kirkpatrick

University of Vermont, Burlington, VT, United States

Typhoid fever causes ~21 million illnesses and up to 600,000 deaths annually. The assessment of new typhoid vaccine efficacy is difficult, because no correlate of protection has been identified. We have developed several assays to test the function of *S.* Typhi-specific antibodies generated following vaccination with a next-generation typhoid vaccine M01ZH09 (*S.* Typhi Ty2 Δ*aroC* Δ*ssaV*) by Emergent Biosolutions. Using an opsonophagocytosis assay, we found that post-vaccination sera increases the uptake of wild type *S.* Typhi Ty2 by human macrophage-like cells (THP-1) up to 2.3-fold relative to pre-vaccination (day 0) or placebo serum samples (p=0.017). Addition of purified immunoglobulins from post-vaccination serum recapitulates opsonophagocytosis results demonstrating that antibodies are largely responsible for the phenotypes observed. Using microscopy, we verified that more bacteria are internalized by macrophages when opsonized with post-vaccination sera than with day 0 or placebos (2-9.5-fold more bacteria/phagocytosing macrophage). Most

importantly, we discovered that the survival of wild type *S.* Typhi, which generally replicates within human macrophages, is reduced up to 50% when opsonized with post-vaccination sera relative to day 0 or placebo serum samples (p=0.049). We also show that antibodies are generated which can be recognized by complement factors and be used to kill wt *S.* Typhi using a bactericidal assay. We show post-vaccination sera has significantly higher bactericidal antibody titers at day 7 (mean = 2972; p=0.031) and day 14 (mean = 5194, p=0.031) relative to day 0 (mean = 886) or placebo controls (mean = 625 all days). These assays are the first to assess the functionality of post-vaccination antibodies in the protection from typhoid infection. This work may lead to the identification of correlates of protection for typhoid fever and may help identify individuals most at risk of acquiring the disease.

509

EVALUATION OF THE TRADITIONAL AND REVISED WHO CLASSIFICATIONS OF DENGUE DISEASE SEVERITY

Federico Narvaez[1], Douglas Elizondo[2], Gamaliel Gutierrez[2], Maria Angeles Pérez[1], Katherine Standish[2], Andrea Nuñez[3], Angel Balmaseda[3], **Eva Harris**[4]

[1]Hospital Infantil Manuel Jesús de Rivera, Managua, Nicaragua, [2]Sustainable Sciences Institute, Managua, Nicaragua, [3]Departamento de Virología, Centro Nacional de Diagnóstico y Referencia, Ministerio de Salud, Managua, Nicaragua, [4]Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, Berkeley, CA, United States

Dengue, a mosquito-borne viral illness, is a major public health problem worldwide and continues to increase in incidence. Infection with one of the four dengue virus serotypes leads to a range of outcomes, including subclinical infection, undifferentiated febrile illness, dengue fever (DF), life-threatening syndromes associated with fluid loss and hypotensive shock, or other severe manifestations such as bleeding and organ failure. The long-standing World Health Organization (WHO) dengue classification and management scheme has recently been revised, replacing the schema of DF, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) with dengue without warning signs, dengue with warning signs (intense abdominal pain, persistent vomiting, fluid accumulation, mucosal bleeding, lethargy, liver enlargement, or hemoconcentration ≥20%) and severe dengue (SD; dengue with plasma leakage leading to respiratory distress, severe hemorrhaging, hypotensive shock or organ failure). We interpreted SD to include compensated shock based on the dengue case management algorithm in the 2009 WHO Guidelines. We evaluated the old and new classification schemes against clinical intervention levels to determine how each captures disease severity using data collected over five years (2005-2010) of a hospital-based study of pediatric dengue in Managua, Nicaragua. Laboratory-confirmed dengue cases (n=557) were categorized using both of the classification schemes, and by level of care (1-3). Level 1 was out-patient care, level 2 was in-patient care that did not meet criteria for level 3, which included intensive care defined by admission to ICU, hypotensive shock, ventilation, administration of inotropic drugs, or organ failure. We therefore tested the sensitivity and specificity of the new and old classifications for severe dengue to identify level 3 care. Sensitivity and specificity for DSS were 45.9% and 76.7%, respectively; sensitivity and specificity for SD were 99.1% and 67.3%, respectively. We are currently extending the analysis to include dengue-negative febrile illnesses, as the DHF/DSS classification is reported to be specific even without laboratory confirmation of dengue. Among dengue-confirmed cases, the new WHO classification for severe dengue appears to have higher sensitivity and specificity to identify cases in need of heightened care, although it is no longer specific for a particular pathogenic entity.

510

HIGH GENOMIC STABILITY OF CHIMERIC YELLOW FEVER/ DENGUE VACCINE STRAINS PRODUCED *IN VITRO*

Yves Girerd-Chambaz, Nathalie Mantel, Patrice Riou, Jean Lang, **Veronique Barban**

Sanofi pasteur, Marcy l'Etoile, France

Most RNA viruses exist as quasi-species in nature and are known to evolve mainly by accumulation of mutations in their genome. The 17D-204 YF attenuated strain is an exception among these viruses and exhibits a remarkable genomic stability in vitro and in vivo, probably due to the low error-rate of the viral RNA polymerase. This enzyme is also the enzyme that ensures viral replication of chimeric Yellow Fever/Dengue (CYD) vaccine viruses, and thus a high genomic stability of these viruses was expected.

The full genome sequence of the 4 CYD viruses was established at various passage levels, from laboratory lots, to GMP vaccine lots (phase 1, phase 2 and phase 3 lots). Sequencing was performed at each step of the manufacturing process from premaster seed lot (PMSL) to master seed lot (MSL), working seed lots (WSL), bulks lots (BL), and at a late step (BL10). Compared to PMSL, no nucleotide substitution was observed, for any serotype, in BL produced for phase 1, 2 and 3 studies despite a cell substrate change at phase 2 level (use of serum-free adapted Vero cells) and the production scale up. No nucleotide substitution was observed at BL10 of CYD1 and CYD2 phase 1 and 2 lots, compared to the corresponding sequence of the PMSL. Regarding CYD3, 3 nucleotide substitutions were detected. A silent mutation at NS5-73 (C>T) was observed in BL10 of phase 2 lots, and 2 amino-acid substitutions, one at NS4B-177 (L>F) and one at E302 (N>N/D) were detected in BL10 of phase I and 2 viruses, respectively The NS4B mutation was also observed at late non-GMP passages of CYD3, during clone selection of CYD2 candidate and at BL10 of phase 1 CYD4 virus, suggesting that it could be the result of adaptation to cell substrate. No mutation was detected at BL10 of phase 2 CYD4. Suckling mice neurovirulence assay was performed for non-conservative mutations, and no increase in neurovirulence was observed.

511

THROMBOCYTOPENIA IN DENGUE PATIENTS: THE EFFECTS OF BODY TEMPERATURE AND CIRCULATING CD41$^+$CD61$^+$ CELLS

Sansanee Noisakran[1], Nattawat Onlamoon[2], Kovit Pattanapanyasat[2], Hui-Mien Hsiao[1], Kulkanya Chokephaibulkit[3], Guey Chuen Perng[1]

[1]Emory University School of Medicine, Atlanta, GA, United States, [2]Office for Research and Development, Siriraj Hospital, Mahidol University, Bangkok, Thailand, [3]Department of Pediatrics, Siriraj Hospital, Mahidol University, Bangkok, Thailand

Dengue has been recognized as one of the most important vector-borne emerging infectious diseases. Severe form of dengue hemorrhagic fever and/or dengue shock syndrome may occur and if left untreated the death rate can be as high as 50%. Currently, there is no preventive vaccine or anti-viral treatment available. Thrombocytopenia and viremia are prominent clinical features in dengue patients. Prolonged fever is a unique feature in dengue patients and yet its association with circulating immune complexes found on the surface of platelets has not been systematically documented in dengue patients, thus factors leading to these clinical features remain elusive. Systematic investigations on the subject with hospitalized dengue patients were initiated. Daily body temperature (BT) and blood samples were measured and collected, respectively, from 36 dengue patients for 9 days. CBC, dengue virus antibodies, viral load (VL), blood smear, and PBMC were obtained. Morphology of and viral antigen in cells were evaluated on blood smears. High levels of VL and BT were observed at the early time points of specimen collection and

declined over time. Their kinetic patterns correlated well with decreasing numbers of platelets. The nadir of platelets count was noticed at the time that VL was undetectable and BT reached normal. Morphological studies revealed that cells with $CD41^{+}CD61^{+}$ surface marker were likely positive for viral antigen. Further investigation suggested that these cells possessed the characteristics of megakaryocytes. Our results imply that a) body temperature associated with circulating-immune complex may be responsible for the low platelets counts, and b)$CD41^{+}CD61^{+}$ cells with megakaryocytic characteristic feature may directly link to dengue viremia. In conclusion, perfect storm of synergy factors interacting with one another including direct infection of megakaryocytes by dengue virus and clearance of dengue virus containing platelets by immune-complex may account for the observed clinical features in dengue patients.

512

PHYLOGENETIC ANALYSIS OF AN ISOLATE OF DENGUE VIRUS TYPE 2 FROM GUATEMALA, 2009

German Añez[1], Caren J. Chancey[1], Andriyan Grinev[1], Maria E. Morales-Betoulle[2], Maria Rios[1]

[1]United States Food and Drug Administration, Bethesda, MD, United States, [2]Universidad del Valle de Guatemala, Guatemala City, Guatemala

Dengue virus (DENV) (genus Flavivirus, family Flaviviridae) is the most prevalent mosquito-borne virus worldwide. The four DENV serotypes (DENV-1 to -4) infect 50-100 million persons annually mostly in Southeast Asia, the Pacific and the Americas. DENV is asymptomatic in approximately 70% of infections, and its spectrum of disease can range from a mild flu-like fever to severe dengue characterized by hemorrhagic manifestations that can lead to shock and death. Dengue has become a public health problem not only in endemic areas but also in non-endemic countries including the US due to importation. Because of the lack of specific treatment or predictive biomarkers for progression to the severe form of the disease, in which survival depends on supportive care, molecular diagnostic techniques are of capital importance for early detection of DENV infections. Here we report the molecular analysis of an isolate obtained from a serologically confirmed case of uncomplicated dengue from Guatemala, collected during the 2009 dengue epidemic. Blood specimens from the febrile phase were made available to us for further investigation. The sample was subjected to quantitative real-time RT-PCR (qRT-PCR) and viral isolation in mosquito C6/36 cells. qRT-PCR results were positive for DENV-2 and the characteristic cytopathic effect of the virus was observed in infected C6/36 cells. Cell culture supernatant from C6/36 cells also tested positive for DENV-2 by qRT-PCR within the first week of culture. The whole genome of the isolate termed Gua09 was sequenced and identified by phylogenetic analysis as closely related to DENV-2 strains from Nicaragua and to belong to the American/Asian genotype of the virus, which has been circulating in Central America for several years now. To our knowledge this is the first report of a fully characterized DENV human isolate from Guatemala. Genetic characterization of DENV isolates are of relevance for the development of molecular diagnostic tools for early identification of infection and proper care of critical patients.

513

IDENTIFICATION OF NEUTRALIZING AND ENHANCING EPITOPES ON DENGUE VIRUS ENVELOPE PROTEIN

Kristen M. Kahle, Kimberly-Anne Mattia, Benjamin J. Doranz

Integral Molecular, Inc., Philadelphia, PA, United States

Characterizing the binding sites of monoclonal antibodies (MAbs) on target antigens, their 'epitopes', can aid in the discovery and development of new vaccines, therapeutics, and diagnostics. Integral Molecular has generated high-resolution epitope maps for human antibodies against the immunodominant envelope protein (prM/E) of Dengue virus (DENV). MAbs used in this study were derived from different immunogens (natural infections and vaccinations), different disease states (Dengue Fever and Dengue Hemorrhagic Fever), and different exposure times (primary and secondary infections). To obtain detailed MAb epitope maps at the resolution of individual amino acids, we developed a novel technology, Shotgun Mutagenesis Mapping. This approach offers the capability of mapping both linear and conformational epitopes, even for structurally complex proteins such as oligomeric Env proteins. A comprehensive mutation library for DENV serotype 3 prM/E protein was created in which *every* residue of the Env protein was individually mutated, expressed in human cells, and analyzed for its effect on antibody immunoreactivity. For each MAb tested, Shotgun Mutagenesis Mapping identified a comprehensive set of amino acids on DENV3 prM/E that are critical for antibody binding. These residues comprise an epitope map that can be visualized on the prM/E three dimensional protein structure. Our goal is to map epitopes on all four DENV Env serotype proteins, and to determine whether the epitopes are shared by different DENV serotypes, if they contribute to antibody-dependent enhancement of infection, and how they relate to the residues that are required for Env function. We expect that this approach will help define the full range of immunodominant structures on Dengue virus and identify novel enhancing and neutralizing antibody epitopes.

514

EVIDENCE FOR *IN VIVO* SUPPRESSION OF TLR-INDUCED INFLAMMATORY BUT NOT OF B-CELL ACTIVATION DURING DENGUE VIRUS INFECTION IN NON-HUMAN PRIMATES

Carlos A. Sariol[1], Yesseinia Anglero[2], Francheska Rivera[3], Petraleigh Pantoja[3], Idia V. Rodriguez[4], Luis Giavedoni[5], Vida Hodara[5], Jorge L. Munoz-Jordan[6], Teresa Arana[3], Laura J. White[7], Melween Martinez[3], Kristina Abel[8], Edmundo N. Kraiselburd[3]

[1]University of Puerto Rico, UMC-CPRC, Departments of Microbiology and Internal Medicine, San Juan, PR, United States, [2]University of Puerto Rico, San Juan, PR, United States, [3]University of Puerto Rico, UMC-CPRC, San Juan, PR, United States, [4]University of Puerto Rico, UMC, San Juan, PR, United States, [5]Department of Virology and Immunology, Southwest National Primate Research Center, Southwest Foundation for Biomedical Research, San Juan, TX, United States, [6]Molecular Virology and Surveillance Laboratory; Centers for Disease Control and Prevention, Dengue Branch, Puerto Rico, San Juan, PR, United States, [7]The Carolina Vaccine Institute, University of North Carolina, Chapel Hill, NC, United States, [8]Department of Microbiology and Immunology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

Dengue is the second most important arthropod-borne disease after malaria, with 50-100 million cases of DF) and 500,000 cases of DHF/DSS each year. In recent years, key roles in determining the outcome of DENV infection have been assigned to the innate immune response, particularly to the PRR such as TLR 7/8 (TLRs) [6,7,8]; the RIG-I/MDA5 [9,10] and the IFN signaling pathways [9-11]. The role of the innate immune response, particularly of DC and of NK cells at early stages after the infection has become a focus of high interest and debate [16,17,18]. However, little is known about the effect of TLRs in the early innate immunological mechanisms that drive adaptative immune responses to dengue virus *in vivo*. On this work we sought to determine the role of such innate signals on the quality of the anti-DENV immune response *in vivo* in NHP. For this purpose animals were infected or mock infected with DENV-1 (WP-74 strain). One infected cohort was also stimulated with TLR 3 and 7/8 agonists and one cohort received TLR agonists only. TLR agonists abrogate the viremia peak at day 4 after the infection and induce a higher frequency of double positive CD40/CD86 mDC. Coincident we the activation of the mDC we found significant higher serum levels of CXCL-10 in the group receiving only the TLR agonists. On the other hand DENV was able to suppress or counteract the TLR-induced inflammatory effect by inhibiting the activation of mDC and controlling the scrum levels of CXCL-10 and IL-1Ra. Of note, the quantity of total specific anti-DENV antibodies was also significantly higher one month after infection in animals receiving TLR agonists. Finally the stimulation with TLR agonists modifies the quality of the anti-DENV B cells response by inhibiting the

IgG1 class switching and increasing the IgG1/IgG2 ratio. For first time we are showing, in NHP model, a quantitative and qualitative modification of the adaptative immune response by TLRs stimulation and activation of the innate immune mechanisms in the setting of an acute viral infection, particularly after DENV infection.

515

COEVOLUTIONARY IMPLICATIONS OF MOSQUITO CGMP-DEPENDENT KINASE AND MOSQUITO-BORNE FLAVIVIRUSES

Julie A. Keating, Dipankar Bhattacharya, Robert Striker

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

Mosquitoes are efficient transmitters of flaviviruses but the molecular mechanisms required to adapt viruses to mosquito transmission are unknown. We have found that mammalian protein kinase G (PKG), a cGMP-dependent protein kinase, directly interacts with and phosphorylates multiple sites in mosquito-borne flaviviral NS5 proteins. PKG also alters insect behavior in Drosophila, honeybees, and ants by mediating phototactic behavior. PKG specifically phosphorylates mosquito-borne flaviviral NS5 at Thr449, but both tick-borne and mammalian-associated flaviviruses lack a phosphoacceptor at this site. Additional PKG phosphorylations occur in the N-terminal methyltransferase of NS5 from mosquito-borne flaviviruses. Interestingly, while PKG phosphorylates both *Aedes* and *Culex*-transmitted flaviviruses, the primary sequence of mosquito PKGs (especially in the regulatory domains) and viral phosphoacceptors differ between mosquito genera. The overall level of phosphorylation of the methyltransferase domain is higher in mosquito-borne than tick-borne flaviviruses. These phosphorylation differences may be linked to the evolution of flaviviruses in adapting to particular insect vectors. While phosphorylation of flaviviral NS5 by mammalian PKG has been identified, we have isolated *Ae. aegypti* PKG and are investigating if and where mosquito PKG phosphorylates NS5. Since PKG upregulation is associated with increased foraging in non-vector insects, we compared the flight activity of mosquitoes whose PKG was pharmacologically activated with control mosquitoes. We found a 3-fold increase in flight activity while maintaining a diurnal pattern, highlighting the importance of PKG in flaviviral insect vectors. Further studies examining PKG's role in flaviviral vector behavior are ongoing. Overall, PKG phosphorylates multiple sites in mosquito-borne flavivirus NS5. The role of PKG in cellular replication of flaviviruses and insect spread of mosquito-borne flaviviruses will be discussed.

516

CHOLESTEROL IS A RISK FACTOR FOR SEVERE DENGUE

Aubree Gordon[1], Crisanta Rocha[2], Federico Narvaez[2], Douglas Elizondo[3], Andrea Nuñez[4], Maria Angeles Pérez[2], Sheyla Silva[2], Angel Balmaseda[4], Eva Harris[1]

[1]Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, Berkeley, CA, United States, [2]Hospital Infantil Manuel Jesús de Rivera, Managua, Nicaragua, [3]Sustainable Sciences Institute, Managua, Nicaragua, [4]Departamento de Virología, Centro Nacional de Diagnóstico y Referencia, Ministerio de Salud, Managua, Nicaragua

Dengue is a mosquito-borne illness that is a major public health worldwide. Clinical manifestations range from dengue fever (DF) to severe forms of the disease associated with hemorrhagic manifestations, vascular leak, and hypovolemic shock. To investigate the association between serum cholesterol levels and development of severe dengue in laboratory-confirmed dengue patients, we performed a prospective hospital-based study of children in Managua, Nicaragua. All children who presented to the National Pediatric Reference Hospital between August 2005 and February 2010 with suspected dengue were eligible to participate in the study. Demographic data and clinical history were collected at enrollment. Data on signs, symptoms, treatments, and laboratory results were collected systematically. Children were considered dengue-positive if dengue virus was detected by RT-PCR or virus isolation or if acute and convalescent sera demonstrated seroconversion by IgM ELISA or a $\geq$4-fold increase in dengue-specific antibodies. Dengue-positive patients were classified as DF, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) following 1997 WHO guidelines. Fasting blood samples for cholesterol measurements were collected each morning from all hospitalized participants. A total of 774 children participated, of which 557 (72%) were hospitalized. Of these, 184 children were dengue-negative, 235 were classified as DF, 93 as DHF, and 45 as DSS. Mean cholesterol levels on the first day of hospitalization were the highest in dengue-negative patients (101.5 mg/dL) and decreased as severity of illness increased; among dengue-positive patients, mean cholesterol levels were 95.7, 84.6, and 70.4 mg/dL, respectively for DF, DHF, and DSS patients. Significant differences ($p<0.001$) in serum cholesterol level were observed between all dengue disease severity categories as well as when comparing dengue-negative patients with all dengue-positive patients. Similar results were observed when comparing HDL cholesterol levels. In multivariable models, the odds of severe disease among dengue patients was 1.3 times higher per 10-unit drop in cholesterol (95% CI 1.2-1.4) when adjusted for day of presentation, age, and sex. Multivariable longitudinal analysis is currently underway to characterize lipid profile changes over the course of disease progression as well as to investigate the prognostic value of cholesterol testing.

517

PENTOXYFILLINE USE TO MODULATE TUMOR NECROSIS FACTOR IN CHILDREN WITH DENGUE HAEMORRHAGIC FEVER

Doris M. Salgado, Tatiana Zabaleta, Martha Rocio Vega, Carlos F. Narvaez, Jairo A. Rodriguez

Universidad Surcolombiana, Neiva, Colombia

Dengue is the main arthropod-borne viral infection in the world, severe forms become a challenge for the clinician because at present no specific treatment is available and there is an increase in the number cases, complications and mortality. The objective of this study was to establish the efficacy of pentoxyfilline for the modulation of the immune response in pediatric patients with dengue hemorrhagic fever. A prospective randomized double-blind study was developed from April to August 2009. A total of 55 patients with symptoms of dengue according to WHO criteria and serologic confirmation of dengue, were included and assigned into 2 groups who were treated with usual treatment, but in the first group Pentoxyfilline was included, whereas in the second was placebo. Complete clinical monitoring and TNFα serum levels were determined during 3 consecutive days. A statistically significant decrease of TNFα levels in dengue patients treated with pentoxyfilline was found ($p=0.02$), this result was more significant in patients classified clinically as having dengue III ($p = 0.003$). In conclusion, taking into account the role of TNFα in the pathophysiology of dengue, pentoxyfilline is suggested as a cost-effective therapeutic measure during the acute phase of severe dengue leading to reduction of complications and death.

518

VIRAL INTERFERENCE BETWEEN DENGUE-2 AND YELLOW FEVER VIRUSES IN VERO CELLS

Flávia A. Saiki, Emiliana P. Costa, **Benedito A. Fonseca**

School of Medicine of Ribeirão Preto, Ribeirão Preto, S.P., Brazil

Dengue is the most important disease caused by an arbovirus worldwide, especially in tropical and subtropical countries, while yellow fever is an important disease to parts of Africa and South America. Although a safe vaccine against yellow fever has been available for over 60 years, there has been an increase in the number of infected people since the beginning of the 1980s in several South American countries, including Brazil. Even though both viruses can be transmitted by the same vector (*Aedes aegypti*) and share some epidemiological features, such as prevalence in

areas where the vector population is abundant, the urban transmission of yellow fever virus hasn't been detected. The causes for this phenomenon are unclear and several hypotheses have been raised to explain this finding. The present study presents a new hypothesis and investigated a phenomenon known as viral interference, a situation where the infection by a particular virus prevents the infection of the same cells by a different virus. We studied the dynamics of both virus replication when dengue-2 virus (DENV-2) and yellow fever virus vaccine strain 17D (YFV-17D) infected the same culture of mammalian cells (Vero cells). We have investigated this phenomenon in *Aedes albopictus* cells (C6/36 cells) using DENV-2 and YFV-17D, and in that case, it was observed a decrease in YFV-17D replication in C6/36 cells previously infected with DENV-2. In this study, we investigated whether or not the same findings were observed in mammalian cells since the interaction between these two important arboviruses in different cell systems can contribute for the understanding of the different aspects related to biology and epidemiology of these two *Flaviviruses.* Our results show that Vero cells chronically infected with YFV-17D (evidenced after 3 days post-infection by RT-PCR) and infected with DENV-2 (YFV-17D-DENV-2) sustained an intense viral interference on the DENV-2. The same interference on the YFV-17D replication was observed when the cells were first infected with DENV-2 (DENV-2-YFV-17D). For both experiments, the MOI was 0.1. These results show the presence of viral interference between these two different *Flaviviruses* in eukaryotic cells and should help to understand the dengue and yellow fever pathophysiology during co-infections in human beings.

519

SEVERE LIVER INFECTION IN AN ADULT PATIENT WITH DENGUE HEMORRHAGIC FEVER: EVIDENCE FOR A BETTER USE OF THE NEW WHO DENGUE CASE CLASSIFICATION

Patrícia Rolim Mendonça, Rômulo Rebouças Lobo, **Benedito A. Fonseca**

School of Medicine of Ribeirão Preto, Ribeirão Preto, S.P., Brazil

Dengue clinical manifestations range from an acute febrile illness (dengue fever) to severe disease [dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS)]. However, other distinct clinical manifestations have been described, such as central nervous system and liver involvement. Based on this plethora of clinical manifestations, the World Health Organization is suggesting the use of a new dengue case classification. To compare the two disease classification, we describe an unusual clinical course of a patient with DHF and presenting with severe liver dysfunction. A 56-year-old male patient was admitted to a university teaching hospital with a 5-day history of fever, headache, myalgia and malaise. He had no past medical history of note. One day before admission he reported epigastric pain, hematemesis, melena and bleeding gums. On admission, at physical examination he was afebrile, oriented, showed normal vital signs, and presented with a slight maculopapular rash, mild mucosal bleeding, and abdominal tenderness. Initial laboratory tests showed leukocytosis, thrombocytopenia, elevated liver enzymes (AST=6,234 U/L; ALT=5,127 U/L) and bilirubin levels, renal failure (creatinine: 3.9mg/dL) and decreased prothrombin activity. In the following days, the patient developed oliguria, drowsiness and episodes of gastrointestinal bleeding, requiring transfusion of blood components due to anemia and a bleeding disorder. A marked increase in liver enzymes developed with peak values of 16,950 U/L and 8740 U/L for AST and ALT, respectively. CPK was normal (132 units/L). An abdominal computed tomography showed mild ascites and splenomegaly. NS1 detection, IgG and IgM antibodies against dengue virus were positive. Improvement in liver function tests occurred gradually in the following days, but superimposed bacterial infection developed and the patient needed orotracheal intubation and vasoactive drugs. Hepatic involvement is a well known feature of dengue but it is usually mild, and liver dysfunction is only observed in the most severe cases. The mechanism of hepatic damage in dengue is poorly understood but viral infection of hepatocytes, induction of apoptosis and immune mediated hepatocyte injury are all possible answers. We present here a dengue case with a severe hepatitis and renal failure, which would complicate the previous WHO classification but it easily classified as severe dengue in the new classification.

520

PEDIATRIC DENGUE SURVEILLANCE IN COLOMBO, SRI LANKA: ANNUAL SERO-CONVERSION

Hasitha A. Tissera[1], Aravinda M. de Silva[2], A. Dharshan De Silva[3], Nihal Abeysinghe[4], Paba Palihawadana[5], Clarence Tam[1], Sunethra Gunasena[6], Ananda Amarasinghe[7], G. William Letson[7], Harold Margolis[7]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]University of North Carolina School of Medicine, Chapel Hill, NC, United States, [3]Genetech Research Institute, Colombo, Sri Lanka, [4]Ministry of Health, Colombo, Sri Lanka, [5]Epidemiology Unit Ministry of Health, Colombo, Sri Lanka, [6]Medical Research Institute, Colombo, Sri Lanka, [7]Pediatric Dengue Vaccine Initiative, Seoul, Republic of Korea

Dengue virus (DENV) infections have been reported in Sri Lanka for nearly 50 years. Cyclical dengue fever/ dengue hemorrhagic fever (DF/ DHF) epidemics have been a regular phenomenon after 1989. Recent epidemics were reportedly more severe. The worst ever epidemic was reported in 2009 with over 35,000 cases. We established a community-based, enhanced passive fever surveillance with a household enrolment model in Colombo, Sri Lanka to estimate burden of dengue infection in the pediatric population. The study is based in Colombo Municipal area which has a high reported annual caseload. A population census of the study area was conducted at the beginning. An age stratified sample of 800 children $\leq$ 12 years of age were selected proportional to the population of each census block to be followed up over a minimum of one year. During the initial recruitment, finger-prick blood samples were collected onto filter paper discs to determine baseline flavivirus sero-status. A repeat finger-prick sample was collected at 12 months to determine the annual sero-conversion rate. An in-house IgG assay was done in order to measure IgG levels in all baseline and one year follow-up samples. Baseline results indicate an overall flavivirus seroprevalence of 52%. Age stratified seroprevalence range from 14% among infants to 72% in children aged 12 years. A preliminary analysis of the first year results shows that 12% of children in the cohort were infected during the follow-up period. For every clinically apparent DENV infection there appears to be two asymptomatic infections. This is the first community based follow-up study in Sri Lanka to estimate burden of dengue infections among children. These results demonstrate the disease endemicity and intense transmission of DENV infection among children in this study area. The study results would be useful in strengthening prevention and control activities of dengue, including dengue vaccine introduction in future.

521

HUMAN DC-SIGN TRANSGENIC MICE AS A MODEL TO STUDY DENV-INDUCED HEMORRHAGE

Betty Wu-Hsieh, Yu-Ting Yen, Ta-Chun Lin, Feng-Chiang Wu, Shin- Yi Jou, Chia-Lin Lai

National Taiwan University College of Medicine, Taipei, Taiwan

In this study, we generated hDC-SIGN transgenic mice in C57BL/6 background. The hDC-SIGN gene was constructed under the polymerase II promoter, followed by a poly(A) tail and two insulators. By RT-qPCR, we showed that the transgenic mice expressed the hDC-SIGN gene in most of the tissues. Flow cytometric analysis showed that around 30-50% of monocytes and peritoneal macrophages expressed hDC-SIGN. Intradermal inoculation of dengue virus (DENV) strain 16681 induced hemorrhage development in hDC-SIGN transgenic mice and the transgenic mice were more susceptible to DENV-induced hemorrhage. To investigate the mechanism of how hDC-SIGN transgenic mice are more susceptible to DENV-induced hemorrhage, we found that macrophages from the transgenic mice produced significantly higher levels of TNF-α

after stimulation by DENV than macrophages from the wild type mice. Furthermore, treatment with neutralizing antibody against TNF-α significantly reduced the incidence and the severity of hemorrhage in transgenic mice. These results together indicate that greater susceptibility of hDC-SIGN transgenic mice to DENV-induced hemorrhage is due to higher TNF-α production by the macrophages in the transgenic mice. The hDC-SIGN transgenic mice will be very useful for testing new drugs and vaccines against DENV.

522

SURVEILLANCE OF DENGUE VIRUS IN FIELD-CAUGHT *AEDES AEGYPTI* FROM THE FLORIDA KEYS BY REAL-TIME RT-PCR

Catherine A. Pruszynski

Florida Keys Mosquito Control, Key West, FL, United States

Dengue Fever and Dengue Hemorrhagic Fever infect as many as 100 million people yearly, and is a significant cause of illness and death in the tropics and subtropics. Though dengue is endemic to South and Central America as well as the Caribbean islands, it rarely occurs in the United States. However, in Fall of 2009, the Florida Department of Health confirmed 22 cases of locally-acquired dengue in Key West, Florida. The prevention and control of dengue relies on the surveillance of circulating virus in the human population and its vector *Aedes aegypti*. Due to the asymptomatic nature of the disease and the high number of tourists traveling throughout the Florida Keys, human serological data are difficult to obtain. A more consistent method for surveillance is the detection of virus RNA in field-caught *Aedes aegypti* mosquito pools. From June through October 2010, mosquito pools were collected from BG Sentinel traps throughout the Florida Keys and assayed by real-time reverse transcriptase polymerase chain reaction for dengue viral RNA. Positive pools were confirmed by the Florida Department of Health Laboratory in Tampa, FL. Results indicate that Key West has the highest prevalence of circulating dengue in *Ae. aegypti* mosquitoes compared to those trapped in Marathon and Key Largo. Vector surveillance through real-time RT-PCR remains a reliable method for dengue detection, and is necessary in order to implement immediate and effective control strategies.

523

COMPARISON OF NEUTRALIZING AND ENHANCING TITERS OF PATIENT AND VACCINEE SERA USING A HIGH-THROUGHPUT DENGUE REPORTER VIRUS DETECTION SYSTEM

Kimberly-Anne Mattia, Elizabeth Christian, Bridget A. Puffer, Benjamin J. Doranz

Integral Molecular, Inc., Philadelphia, PA, United States

The lack of reliable, high-throughput tools for characterizing anti-Dengue virus (DENV) antibodies in large numbers of serum samples has been an obstacle in understanding the impact of neutralizing and enhancing antibodies on disease progression. DENV reporter virus particles (RVPs) have been developed to facilitate the genetic manipulation and biological characterization of DENV virions. RVPs are produced by combining a subgenomic replicon encoding an optical reporter with structural components from each of the four defined serotypes of DENV. RVP infection is monitored by expression of the reporter gene using standard optical detection platforms. RVPs are antigenically equivalent to wild-type virions but lack the viral machinery required for a productive infection, making them a safe reagent to rapidly assess humoral immune responses to all four serotypes. In this study, we demonstrate the diagnostic utility of DENV RVPs for characterizing antibodies in human serum samples. RVPs expressing either GFP or luciferase reporters were tested for optimal detection of infection, serotype-specific neutralization, and enhancement of infection. We assessed the suitability of RVPs for long-term, large-scale studies by optimizing storage conditions, and testing the reproducibility of infection in different cell lines. Neutralization titers obtained using RVPs were statistically identical to those derived using the plaque reduction neutralization test (PRNT). Finally, RVPs were used to identify and score sera from individuals vaccinated with live attenuated DENV and from patients naturally exposed to DENV. Comparison of sera neutralization and enhancement allows for a more complete antibody profile, documenting the potentially protective and pathogenic humoral immune response against each serotype of DENV within each patient. These experiments validate DENV RVPs as a high-throughput reagent for measuring neutralizing and enhancing antibody responses, and present a novel tool for understanding the effects of natural infection and vaccination on large patient populations.

524

LONG-TERM DOMINANCE OF DENGUE VIRUS TYPE II PUERTO RICAN LINEAGES THROUGHOUT MAJOR EPIDEMIOLOGICAL CHANGES

Gilberto A. Santiago[1], Kate L. McElroy[1], Niall J. Lennon[2], Bruce W. Birren[2], Matthew R. Henn[2], Jorge L. Muñoz-Jordán[1]

[1]Centers for Disease Control and Prevention, San Juan, PR, United States, [2]Broad Institute of MIT and Harvard, Cambridge, MA, United States

Previous studies of dengue virus serotype 2 (DENV-2) Asian/American genotype in Puerto Rico had documented positive selection, rare introductions and a clade replacement prior to its drastic decline in the early 2000's. The virus re-emerged in 2003 and has become the only of the four DENV serotypes to have been uninterruptedly transmitted on the island since the early 1980's. We re-examine the evolution of the autochthonous, Puerto Rican DENV-2 over a period of 22 years by conducting full genome sequencing of an expanded sampling of 160 genomes, including 53 from the 2002-2007 period covering the decline and re-emergence of DENV-2. Although our phylogenetic analysis confirms lineage turn-over events, we show that DENV-2 evolves through a strong, negative, purifying selection, and document new findings of epidemiological importance. Puerto Rican DENV-2 lineages are defined by temporal and geographical associations and the virus persisted in a restricted circumscription through 3 years of extreme paucity, to then re-emerge and disperse across the island. We also re-examine the role of re-introductions in the epidemiology of DENV-2 in Puerto Rico and find that foreign strains are frequently but transiently transmitted across periods of high DENV-2 dominance; but rarely displace the autochthonous virus. All together, our analyses indicate superior fitness of the Puerto Rican DENV-2 lineages, which may explain its long term endurance despite great epidemiological changes.

525

IMPLEMENTING A SEXUAL ASSAULT TRAINING FOR NURSES AT JFK MEMORIAL CENTER IN MONROVIA, LIBERIA

Abiola A. Fasina

Mount Sinai School of Medicine, New York, NY, United States

Liberia's first civil war lasted from 1989 till 1997. Violence erupted again in the second civil war from 1999 till 2003. Qualitative data from these periods indicates high rates of sexual assault, coercion and rape. The United Nations Mission now maintains peace in Liberia but women are still experiencing the impact of gender based violence (GBV) from during and after the war and there are few resources to care for survivors. At present, Medecins Sans Frontieres (MSF) is providing services but is planning to leave Liberia in June, 2010. The purpose of this project was to train health care providers at JFK Memorial Center (JFK-MC) in the capital city of Monrovia to provide care for survivors of GBV when MSF leaves. Adapting a training tool developed by the International Rescue Committee (IRC), a curriculum was developed for nurses who would be caring for survivors of GBV. The purpose was to familiarize the nurses with international standards and appropriate referrals for survivors. This 3 day training consisted of case based scenarios, review of clinical management, role play and a DVD reviewing the various components of the exam with actors demonstrating key points. A post training survey was administered

to participants. Nurses were selected by senior nursing management from the departments of obstetrics, emergency medicine, internal medicine and the trauma service. The project was reviewed and approved by the hospital's general administrator who functions as the chief managing officer. 15 nurses participated in the training. The proportion of nurses who were 'very comfortable' providing medical care for survivors of sexual assault increased from 14% to 93% as a result of the training. Paired T-test (SPSS 17) indicated that this change was significant ($p < 0.01$). The proportion that felt very comfortable with counseling and referral increased from 29% to 71%. This change was also significant by paired T-test (SPSS 17, $p < 0.05$). 93% of participants rated the training as very good or satisfactory. In conclusion, training programs can improve knowledge and ability to care for survivors of GBV.

526

DIARRHEA MORTALITY IN CHILDREN AGED 5 TO 14 YEARS IN INDIA

Shaun K. Morris[1], Diego G. Bassani[2], Shally Awasthi[3], Rajesh Kumar[4], Prabhat Jha[2], for the MDS Collaborators[5]

[1]The Hospital For Sick Children, Toronto, ON, Canada, [2]Centre for Global Health Research, Li Ka Shing Knowledge Institute, St. Michael's Hospital, Toronto, ON, Canada, [3]Department of Pediatrics, King George's Medical University, Lucknow, India, [4]School of Public Health, Postgraduate Institute of Medical Education and Research, Chandigarh, India, [5]India

Diarrheal diseases are a leading cause of death in children under the age of 5. However, very little is known about diarrhea mortality in children over the age of 5. Our objective is to provide the first nationally representative estimate of diarrheal mortality in Indian children aged 5 to 14 years and to understand the distribution of these deaths based on geographic region, age, and gender. This study uses data from the verbal autopsy based Million Death Study (MDS). The MDS surveyed 6.3 million people in 1.1 million nationally representative Indian households for vital status between 2001 and 2003. Diarrheal deaths were defined ICD-10 codes A00 and A02-A09. Estimates of number of deaths were obtained by applying the proportion of deaths by gender, age, and state caused by diarrhea from the MDS to the corresponding UN Population division derived death envelopes for India in 2005. We estimate there are approximately 45,000 annual deaths due to diarrhea in children aged 5 to 14 years in India. The mortality rate is approximately 35% higher for girls than in boys for both age groups 5 to 9 years and 10 to 14 years. There were significant differences by region; the diarrhea mortality rate in children older than 5 ranged from a high of 39.3 per 100,000 in the Northeast to a low of 3.6 per 100,000 in the South. In conclusion, there are no existing estimates for the burden of diarrhea mortality in older Indian children, however, our estimate of 45,000 annual deaths is significantly larger than the Global Burden of Disease estimate of approximately 1,000 deaths in children aged 5 to 14 years in all of South Asia. Additional research is needed to better understand the etiologic causes of these illnesses as well as underlying risk factors for death. The large gender and geographic differences in mortality rates suggest the potential for significant numbers of lives saved though strengthening health education and access to health services.

527

HEALTH PROBLEMS AMONG JAPANESE EMBASSY PERSONNEL IN HANOI, VIETNAM

Yasutaka Mizuno[1], Thi Bach Duong Hoan[2]

[1]National Center for Global Health and Medicine, Tokyo, Japan, [2]Embassy of Japan in Vietnam, Hanoi, Vietnam

The number of Japanese expatriates has increased in recent years through internationalization. According to data from the Ministry of Foreign Affairs of Japan, there were 106 million Japanese expatriates in the world in 2006. Although many studies about health problems among short-term travelers have been conducted, relatively few reports exist on expatriates who reside for extended periods of time in developing countries. The objective of this study is to evaluate health problems among Japanese expatriates in Vietnam, and to clarify whether there were differences in the illnesses reported by Japanese expatriates and local people who were examined at the medical division of the Japanese embassy in Vietnam. Data on embassy personnel, their dependents, and local employees with any health problems, who visited the medical division in the embassy from May 2007 to April 2008, were retrospectively collected. The patients were examined by a medical attache in order to make a clinical diagnosis. According to these reported diagnoses, we evaluated the difference of health problems between Japanese and Vietnamese personnel. A total of 696 patients visited the medical division for the purpose of medical consultation, laboratory examination, and vaccinations and because of health problems. Of these, 421 (60.5%) were Japanese. The mean age was 33.95 ± 12.11 years. The remaining 275 (39.5%) patients were Vietnamese. The mean age was 39.56 ± 7.98 years. The most frequent purpose of visit was due to respiratory problems (n=188), followed by vaccinations (n=111), and due to gastro-intestinal (G-I) problems (n=96). G-I and eye problems were more frequently seen in Japanese, whereas genital, orthopedic, and skin problems were more frequently seen in Vietnamese. In conclusion, although health problems among Japanese expatriates seem similar to short term travelers, some illnesses are possibly related to the local environmental situation. Additionally, there were slight differences of illnesses between Japanese expatriates and Vietnamese local residents. These results indicate that differences in health problems might have been due to culture and lifestyle differences. Thus, in order to provide appropriate expatriate health care, travel health practitioners should consider not only disease prevalence but also the environmental and cultural situation of a country.

528

WHAT DOES IT COST TO ASSESS TRACHOMA PREVALENCE?

Chaoqun Chen[1], Elizabeth A. Cromwell[2], Paul M. Emerson[2], Jonathan D. King[2]

[1]Georgia Institute of Technology, Atlanta, GA, United States, [2]The Carter Center, Atlanta, GA, United States

Trachoma prevalence surveys are used to map the disease, for planning trachoma control program implementation, and to assess program impact. Many national programs have not conducted trachoma prevalence surveys because of the perception that they are too expensive. We analyzed the cost and the factors that influence cost the most (cost drivers) for 193 surveys from seven national programs.

Actual field costs were collected from the national programs and the country offices of the implementing agencies for trachoma prevalence surveys conducted from 2006 to 2010. Surveys included in this analysis used a cluster random sampling design with15-24 clusters surveyed per domain. Data were reviewed for accuracy and checked against financial reporting records. Costs were converted to USD using the three year average exchange rate. The median cost per district (domain) surveyed was $4,738 (Inter Quartile Range [IQR]: $3,315-$5,898) and the median cost per cluster was $291 (IQR: $110-$356). The main cost drivers were: field work (per diem and accommodation for the survey team) which accounted for 41.3% of all costs; transport (driver per diem, fuel and associated costs of running a program vehicle, and vehicle rentals), 18.9%; and data entry, 10.1%. These data can be used to project future prevalence survey costs in areas not yet mapped for trachoma.

529

IMMUNE SYSTEM DEVELOPMENT DURING EARLY CHILDHOOD IN TROPICAL LATIN AMERICA: EVIDENCE FOR THE AGE-DEPENDENT DOWN REGULATION OF THE INNATE IMMUNE RESPONSE

Philip Cooper[1], Rommy Teran[1], Maritza Vaca[1], Silvia Erazo[1], Gisela Oviedo[1], Martha Chico[1], Marc Huebner[2], Quentin Bickle[3], Laura C. Rodrigues[3], Edward Mitre[2]

[1]Laboratorio de Investigaciones FEPIS, Quito, Ecuador, [2]Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [3]London School of Hygiene and Tropical Medicine, London, United Kingdom

There are important differences in the prevalence of inflammatory diseases such as asthma between populations living in urban and rural areas of tropical Latin America (LA). The immune response that develops in early childhood is considered to underlie the development of such diseases but there are few data. The present study investigated the effects of age and environment (urban vs. rural) on the development of immunity during the first 5 years of life in tropical LA. A cross-sectional study was conducted of Afro-Ecuadorian children aged 6-9 months, 22-26 months, and 48-60 months living in urban and rural Esmeraldas Province in Ecuador. Data was collected by parental questionnaire and blood samples were collected to measure innate and adaptive immunity. Data obtained clearly demonstrated that the immune system is actively developing throughout the first five years of life as frequencies of naïve CD4+ T cells declined with age while those of memory CD4+ and CD8+ T cells increased. Infants aged 6-9 months had evidence of hyper-reactive innate immune responses to TLR agonists compared to older children. Regulatory responses including T-cell production of IL-10 and frequencies of FoxP3+ T-regulatory cells decreased with age. No substantial effects of environment were observed on these innate and adaptive immune responses. These results suggest that innate immune responses decline with age during early childhood in tropical LA in parallel with declines in regulatory responses. Enhanced innate immunity in early life may be important for host defense against pathogens but may increase the risk of immunopathology.

530

ONE HEALTH INITIATIVE: A FOCUS FOR CROSS-SPECIES MEDICINE

Jack Woodall, Thomas P. Monath, Laura H. Kahn, Bruce Kaplan

One Health Initiative, Sarasota, FL, United States

The One Health Initiative is an internet forum promoting co-equal, all inclusive collaboration between physicians, veterinarians and professionals in health related disciplines, endorsed by major medical organizations and health agencies, including the American Medical Association, American Veterinary Medical Association, American Society of Tropical Medicine and Hygiene, U.S. Centers for Disease Control and Prevention (CDC), Association of American Medical Colleges, Association of American Veterinary Medical Colleges, Association of Schools of Public Health, American Society for Microbiology and many others. More than 500 prominent scientists, physicians and veterinarians worldwide have endorsed the initiative. The One Health concept is a worldwide strategy for expanding interdisciplinary collaboration and communication in all aspects of health care for humans and animals. The synergism achieved will advance health care for the 21st century and beyond by accelerating biomedical research discoveries, enhancing public health efficacy, expeditiously expanding the scientific knowledge base, and improving medical education and clinical care. When implemented it will protect and save untold millions of lives in our present and future generations. Recognizing that human and animal health are inextricably linked, One Health seeks to promote, improve, and protect the health and well-being of all species by enhancing cooperation and collaboration between physicians, veterinarians and other scientific health professionals, and by promoting strong leadership and management to achieve these goals. The vision of the One Health Initiative is to serve the medical, veterinary medical, nursing, public health and environmental health communities by providing a forum for the exchange of One Health research findings and innovative ideas in education, clinical care and public health. The ultimate goal is to improve the health of all species including humans, domestic animals, wildlife and plants. The authors are independent of any other entity or organization; we support and augment efforts of other organizations to recognize, promote and implement One Health. This autonomous endeavor is sustained *pro bono* due to our firm conviction regarding the enormous value of the "One Health/One Medicine" concept.

531

MEASURING CHILD PSYCHOSOCIAL STIMULATION BY CAREGIVERS IN THE DOMINICAN REPUBLIC

Jessica E. Sjoblom, John D. McLennan

University of Calgary, Calgary, AB, Canada

Child psychosocial stimulation by caregivers during the early years has an important role in the development of children. However, there are few reports on these practices from low-and middle-income countries (LMICs) and few feasible instruments to measure these practices. This study (i) describes caregiver psychosocial stimulation practices in a peri-urban community in the Dominican Republic, (ii) examines socio-demographic predictors of these practices, and (iii) determines the test-retest reliability of the Family Care Indicators (FCI), a tool to measure psychosocial stimulation. Caregivers of young children (n=220) living in a peri-urban district on the outskirts of Santo Domingo, Dominican Republic participated in a structured interview. The interview included items from the FCI and additional questions regarding play materials and caregiver engagement with children in play activities. Socio-demographic predictors were examined using regression analysis. Test-retest reliability of FCI items between baseline and follow-up interviews was determined. Typical play materials of children were store bought and both play materials and activities of children usually involved pretend play and movement. Socio-demographic predictors of practices included child and caregiver age, number of children of the caregiver, and caregiver's level of educational attainment. Test-retest reliability of FCI items ranged from moderate to good. In conclusion, in order to increase the reliability of the FCI, some items may need to be reworded to reduce ambiguity. Inclusion of items on pretend play may improve the scope of the instrument to better capture a more diverse range of important psychosocial stimulation practices. More research is necessary to determine the utility of the FCI, or a modified FCI, in quantifying stimulation practices cross culturally.

532

IN SEARCH OF COMPREHENSIVE HEALTH CARE: BIOMEDICINE AND BEYOND

Catherine M. Sebuguzi[1], Florence Nankya[1], Joaniter Nankabirwa[1], James Kizito[1], Christine Nabirye[1], Clare Chandler[2], Sarah G. Staedke[2]

[1]Infectious Disease Research Collaboraton, Kampala, Uganda, [2]London School of Hygiene and Tropical Medicine, London, United Kingdom

Formal health facilities, focusing on delivery of biomedical treatment, are one of many sources of care for febrile patients in sub-Saharan Africa. To increase prompt access to appropriate biomedical treatment, rationales behind current treatment-seeking must be understood, and any gaps in the services offered by the formal sector must be considered as areas for improvement. We carried out a study of community perceptions, preferences and experiences in treatment seeking in Tororo district, Eastern Uganda. We carried out 10 focus group discussions with community members, including primary care givers (n=5) and household heads (n=5). We triangulated the findings with in-depth interviews with 100 community medicine distributors who acted as key informants with insight into treatment-seeking patterns.

In this area, first-line treatment for most conditions was with a biomedical drug. Sources of these drugs included government-run health centers, private clinics and drug shops. Nearly all community members had visited their local health centre, but dissatisfaction with experiences there was high. Herbal medicine was frequently used and interestingly, community members also relied heavily on shrines, churches and prayers for treatment. Choice of health care was influenced by the following factors: (1) initial perceptions and beliefs about etiology and severity of the illness that would, from experience, require a particular source of treatment. Often, experience showed health centers to be a poorer source of care than other providers for common illnesses; (2) accessibility of the preferred treatment, which relied on distance to the provider as well as opening hours, spousal support in meeting costs, opportunity costs of leaving the home and travelling to the provider, ability to negotiate the logistical and social rules of the provider's institution, and availability of treatment at that provider; and (3) trial and error in moving between treatment sources.

Our results suggest that care from health facilities frequently does not meet patients' expectations. Biomedical drugs were valued as a first port of call, but the wider process of care at health centers was unsatisfactory, leading patients to seek care from alternative, non-medical sources. Interventions designed to improve health care delivery need to attend to the wider needs of patients beyond the biomedical paradigm of pharmaceutical treatment.

533

MAPPING THE GLOBAL DISTRIBUTION OF TRACHOMA: AN UPDATED ATLAS

Jennifer Smith[1], Simon Brooker[1], Danny Haddad[2], Sarah Polack[1], Pamela Hooper[2], Emma Harding-Esch[1], Robin Bailey[1], David Mabey[1]

[1]London School of Hygiene and Tropical Medicine, London, United Kingdom, [2]International Trachoma Initiative, Task Force for Global Health, Atlanta, GA, United States

With the aim of eliminating trachoma as a cause of blindness by the year 2020, it is increasingly important to finely target and coordinate strategies to control this focal disease. A geographically targeted approach relies on the availability of reliable and updated maps of trachoma prevalence. Although the number of surveys conducted has increased in recent year, the data are rarely consolidated or presented in a form easily accessible to policy makers, programme managers and potential donors. A previous trachoma atlas developed in 2005 by the London School of Hygiene and Tropical Medicine collated information at a district level, and there is now a need to update this atlas and allow for changing administrative boundaries. We describe the search strategy and assembly methods for development of an updated, open-access, *Global Atlas of Trachoma*. Estimates of active trachoma and trichiasis were disaggregated to the village level to enable detailed mapping of disease distribution and a flexible approach in calculating prevalence estimates. Details of survey population, diagnostic methods, sample size and numbers examined and infected were abstracted into a single database, and all surveyed locations assigned to a specific longitude and latitude using standardized geolocation procedures. Population coverage of different SAFE interventions was included in a linked database. Based on information assembled in the updated atlas, we quantify the current geographical distribution of trachoma and evaluate progress in control. Information sources date from 1985 and include surveys conducted by ministries of health and NGO partners, as well as academic research studies. Currently, 46 countries have district-level data included and follow-up is ongoing for disaggregated data. Three countries have nationwide surveys and an additional 13 have substantial geographic coverage of endemic areas. The majority of data are from population based surveys (70%), but rapid assessment (20%) and other sampling methodologies are also represented. It is envisioned that these maps will have important applications in quantifying the global burden of trachoma and targeting of future control efforts.

534

PRICE MARK-UPS AND PRICING DETERMINANTS OF ARTEMISININ-COMBINATION THERAPY (ACT) IN THE PRIVATE COMMERCIAL SECTOR DISTRIBUTION CHAIN FOR ANTIMALARIAL DRUGS IN CAMBODIA

Edith Patouillard[1], Benjamin Palafox[1], Sarah Tougher[1], Sochea Phok[2], Duong Socheat[3], Kate OConnell[4], Immo Kleinschmidt[1], Kara Hanson[1], Catherine Goodman[1]

[1]London School of Hygiene and Tropical Medicine, London, United Kingdom, [2]Population Services International, Phnom Penh, Cambodia, [3]National Centre of Entomology, Parasitological and Malaria Control, Phnom Penh, Cambodia, [4]Population Services International, Nairobi, Kenya

In many developing countries, the private commercial sector is an important provider of malaria treatment. To design effective interventions for improved malaria control, there is a need to understand retailers' behaviour and identify the factors influencing their stocking and pricing decisions. Retailers are the last link in a chain of wholesalers and retail outcomes are likely to be influenced by what happens further up the chain. However, little is known about the retail sector distribution chain and its influence on the availability and prices of antimalarials that consumers can access. This study is part of ACTwatch, a multi-country project that aims to provide evidence to policymakers on the use, availability and prices of quality malaria treatment. In Cambodia, nationally representative samples of retailers and wholesalers selling antimalarials were surveyed: 1127 structured interviews and 27 in-depth interviews with providers operating at different levels of the distribution chain collected data on providers' characteristics, and stocking and pricing behaviour for antimalarials. Data collection took place during the malaria transmission risk season between June and November 2009. Median percentage mark-ups on ACT ranged at retail level between 33.5% in village shops and 50.0% in drug shops, and at wholesale level between 39.9% at the level supplying retailers and 39% at that supplying higher levels of the distribution chain. In absolute terms, median mark-ups on one adult equivalent treatment dose amounted to US$0.50 in village shops, US$0.68 in drug shops, US$0.18 at the level supplying retailers and US$0.14 at the level supplying higher levels. Whilst there was no evidence of a difference in percentage mark-ups for ACT between retail and wholesale levels ($p= 0.882$), there was strong evidence that absolute mark-ups at retail level exceeded mark-ups at wholesale levels ($p<0.0001$). Findings will also be presented on the influence on ACT pricing of structural aspects of the distribution chain and relationships between providers.

535

A GLOBAL MAP OF RESEARCH AND DEVELOPMENT FOR MALARIA - WHO IS DOING THE WORK?

Mary Moran, Javier Guzman, Alina McDonald, Klara Henderson

The George Institute for International Health, Sydney NSW, Australia

Over $US 1 billion was spent on research and development (R&D) of new malaria products in 2007 and 2008, but there is limited information on how funds were invested. We have therefore analysed these malaria funding recipients by organisation type, location and type of research. Unpublished data from the G-FINDER survey has allowed us to create a map of the malaria R&D workforce. Seventy percent (US$760M) of 2007 and 2008 malaria R&D funding was disbursed to over 250 recipients including public researchers, small biotechnology companies (SMEs) and multinational pharmaceutical companies (MNCs) in more than 30 countries, half being developing countries. Twenty percent (US$250M) was disbursed to Product Development Partnerships (PDPs) and other intermediaries. Over two thirds was received by organisations in the USA (32%), UK (21%) and Switzerland (15%). If funding to PDPs and intermediaries is excluded - since this is mostly disbursed on to third organisations - organisations based in the USA and UK still received nearly half of all funding (22% and 20%). Basic research received almost 25%

of funds, and product development around 40%. Public institutions represented 75% of basic research funding; discovery and preclinical research were conducted by MNCs (36%), public institutions (33%), PDPs (12%) and SMEs (9%); while PDPs and MNCs led clinical development (around 40% each). Public groups were previously primarily responsible for discovering new malaria product leads, taking these through to Phase II. Government incentives then sought (largely unsuccessfully) to encourage industry to take leads through clinical development to registration and large-scale manufacture. Today, while public groups continue to generate vital basic research, it is companies and PDPs who are primarily responsible for discovering leads and taking these through early clinical trials, while PDPs also manage 40% of clinical development investment. It is not clear that policy settings have kept up with this shift in activity.

536

ENCOURAGING PRIVATE SECTOR INVOLVEMENT THROUGH ELIMINATION OF IMPORT TARIFFS ON ANTI-MALARIA MEDICINES

Halima Mwenesi, **A. Elisabeth Sommerfelt**, Kenyetta Lovings

Academy for Educational Development, Washington, DC, United States

The United Nations (UN) has emphasized the need for multi-sectoral involvement for successful control, elimination, and eventual eradication of malaria. In addition to contributions from international donors and public sector engagement, this requires large-scale involvement of various actors in the private sector for sustained coverage with effective interventions. When import tariffs are eliminated, the likelihood of private sector participation increases, keeps prices low and universal access high. International fora (including the UN General Assembly, World Health Assembly and various regional commitments) have called for the elimination/reduction of taxes and tariffs on anti-malaria tools/interventions (including treated bednets, medicines used with rapid diagnostic tests, and indoor residual spraying). Based on our extensive analysis of data on tariffs reported by countries, ~1/3 of countries identified by Roll Back Malaria as having a substantial malaria problem still impose tariffs on all these interventions. For information on the private sector as a source of care for under-five children with recent fever, we analyzed nationally representative survey data from the Demographic and Health Surveys Program (DHS). Among countries that impose tariffs on all these interventions, we selected those with recent DHS surveys (from 2007) and a substantial malaria problem (D.R. Congo, Indonesia, Philippines, Sierra Leone) (sample sizes of children ranged from ~5000 to ~16000), analyzing sources of care reported by mother. Illustrative findings from the Indonesia 2007 DHS show that care was sought for 9 of 10 children with fever; ~20% received care from a public facility and almost 60% from a private facility/provider. There were differentials according to poverty-wealth quintile for care received from public (~25% of poorest vs. slightly above 10% of richest) and private (almost 50% of poorest vs. almost 75% of richest) sources. These findings demonstrate that the private sector is an important source of care for fever (and malaria) even for the poorest subgroups.

537

ACCEPTABILITY OF TRANSPORT COST PAYMENT FOR MOTHERS SEEKING ANTENATAL AND DELIVERY CARE SERVICES: A SURVEY OF TRANSPORTERS' ATTITUDES - EASTERN UGANDA, 2009

Moses Tetui, Elizabeth K. Ekirapa, John Bua, Aloysius Mutebi, Raymond Tweheyo, George W. Pariyo

Makerere University College of Health Sciences, School of Public Health, Kampala, Uganda

Geographical accessibility is a major limitation to utilisation of maternal health services in Uganda. This study explored; transport availability, perceptions of subsidising transportation costs for antenatal (ANC) and delivery care services in four districts of Eastern Uganda. This was a cross-sectional study using qualitative approaches, carried out in Tororo, Pallisa, Soroti and Kamuli districts of Eastern Uganda in October 2009. Key informant interviews and focus group discussions were held with bicycle and motorcycle riders, and their transport leaders. Thematic content analysis was used. Findings revealed that mothers had great difficulties in accessing ANC and delivery services partly because of lack of transport. Most mothers who sought the services walked to health facilities, although public bicycles, motorcycles and vehicle transport means were available for their use. Service users were often unable to afford the transport charges. The project to help the mothers was generally acceptable in all the four study districts. Majority of the transport providers preferred weekly cash payments to be made either to them or their leaders rather than through bank accounts. The transporters acknowledged that project implementation could encounter potential challenges such as; insecurity at night, conflicts with non-beneficiaries (those who are not pregnant), conflicts with husbands, lack of night duty midwives, delays at the health centres, poor communication, lack of ownership of transport means, delayed payments, determining payments during day and night. In conclusion, findings suggest that subsidizing transportation for ANC and delivery care for pregnant women was acceptable, however, with potential social and institutional challenges. The study recommends accommodation of stakeholder suggestions in the project planning cycle, involvement of local authorities and community sensitization through various media.

538

NORTH-SOUTH COLLABORATIONS IN NON-COMMERCIAL CLINICAL RESEARCH: OPEN CHALLENGES

Raffaella M. Ravinetto[1], Ambrose Talisuna[2], Halidou Tinto[3], Umberto D'Alessandro[1], 4 Artemisinin-Based Combinations Study Group[4]

[1]Institute Tropical Medicine Prince Leopold, Antwerp, Belgium, [2]Uganda Malaria Surveillance Project, Kampala, Uganda, [3]Clinical Research Unit, Centre Muraz, Nanoro, Burkina Faso, [4]Multicentre, Antwerp, Belgium

North-South collaborations in clinical research have a double goal: addressing public health needs and building capacity and co-ownership. The 4-ABC trial, Evaluation of 4 artemisinin-based combinations for treating uncomplicated malaria in African children (ClinTrialGov NCT00393679) was funded by EDCTP, sponsored by the Antwerp Institute of Tropical Medicine (ITM) and conducted in Burkina Faso, Gabon, Mozambique, Nigeria, Rwanda, Uganda and Zambia. Protocol and amendments were sequentially submitted to the ITM Institutional Review Board, Ethics Committee (EC) of Antwerp University Hospital and EC and Competent Authorities in host countries. 4114 patients were recruited in 10 sites; data were recorded via an electronic CRF, cleaned at ITM and analyzed in Liverpool with the participation of an African statistician. PCR were read at ITM with the participation of an African biologist. The study group, co-chaired by an ITM and an African malaria expert, faced context-related, budgetary and structural constraints. In externally-funded trials, budgets tend to be inadequate to fully comply with GCP formal requirements and promptly react to unexpected situations. In addition, North-South non-commercial consortia have limited structural resources for clinical research tasks (data management, pharmacovigilance, regulatory affairs). Specific difficulties were linked to multiple ethical reviews; harmonization of QC procedures; samples' shipment; staff retraining; safety reporting; rationalization of monitoring expenses; cultural adaptation of informed consent in various urban and rural contexts; shelf life extension/discontinuation of two study drugs; set up of off-line remote data entry system; lack of insurers in host countries to cover study-specific risks. The capacity building effort brings empowerment, networking and capacity transfer to the South. However, the transfer of capacity to lead and sponsor trials is delayed by persisting obstacles, including the lack of secured funds for local structural costs and the need to translate universal GCP principles in contextualized procedures.

539

ASPIRATIONS FOR QUALITY HEALTH CARE: HOW DO WE GET THERE?

Clare I. Chandler[1], James Kizito[2], Deborah DiLiberto[1], Sarah G. Staedke[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Uganda Malaria Surveillance Project, Infectious Disease Research Collaboration, Kampala, Uganda

Access to 'prompt, effective treatment' is the dominant discourse in policies and programmes to reduce the burden of diseases such as malaria. However, decades of efforts to achieve this through public, private and community-based initiatives have barely dented morbidity and mortality levels in many settings, particularly in rural Africa. We carried out an assessment of perceptions of quality health care and barriers to achieving this in Tororo, Eastern Uganda. We carried out 69 in-depth interviews and 6 focus group discussions with health workers, 100 in-depth interviews with community medicine distributors and 10 focus group discussions with community members across 5 sub-counties in Tororo district. We found that aspirations for good quality care were similar amongst the three groups, and identified the most frequently discussed values: good clinical care and treatment; good interpersonal interactions between health workers and patients; well-managed health centres; providing advice and explanations to patients; welcoming and guiding the patient through health centres; professionalism amongst health workers; provision of free treatment at convenient times; and being seen quickly on arrival at the health centre. At health centres, immediate barriers to quality care included drug stock-outs and lack of equipment; high patient to staff ratio; use of volunteer health workers; language barrier between health workers and patients and discriminatory treatment of patients. Underlying these barriers were poor motivation of staff; poor management of the health centre; lack of patient-centred culture and poor relationship between health workers and communities. We traced these factors to district level determinants, including prioritisation of funds and politicking by district officials, and to wider systemic issues and cultural values. We argue that in order to attract patients to health centres and improve health outcomes, interventions need to build on the values and aspirations of health workers and community members rather than focus on narrow biomedical goals. We will present strategies designed to tackle these wider issues, to be evaluated through a 2-year cluster randomised controlled trial.

540

EFFECT OF TEMPERATURE AND PROINFLAMMATORY CYTOKINES ON PHOSPHATIDYLSERINE EXPRESSION ON *PLASMODIUM FALCIPARUM* MALARIA-INFECTED RED BLOOD CELLS DURING PARASITE MATURATION

Kovit Pattanapanyasat[1], Panudda Sratongno[1], Pattamawan Chimma[1], Supapart Chitjamnongchai[1], Korakot Polsrila[1], Kesinee Chotivanich[2]

[1]Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, [2]Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Malaria caused by *Plasmodium falciparum* remains one of the world's largest burdens of disease. Cerebral malaria is a life-threatening complication believed to be associated with parasitized red bloods (pRBCs) sequestration within the microvasculature of vital organs. Intra-RBC maturation of the malaria parasite corresponds with profound changes in the asymmetry of phospholipids in the lipid bilayer of the pRBCs. These changes may contribute to adherence of pRBCs to endothelial cells. The present study investigates the effect of febrile temperature and pro-inflammatory cytokines usually encountered during symptomatic human malaria infection on phosphatidylserine (PS) expression on the surface membrane of pRBCs during parasite maturation. The expression of PS on the pRBCs was determined by flow cytometry using fluorescence-labeled annexin V, which specifically binds to PS and a vital nucleic acid fluorochrome for parasite staining. The results showed that PS expression on the surface of pRBCs increased in association with parasite maturation, particularly at the late parasite stage. Exposure to febrile temperature led to significant increases in the expression of PS on the surface of pRBCs, especially at the late parasite stage associated with the virulence strain of the parasite. In contrast, pro-inflammatory cytokines had no detectable effect on PS expression on pRBCs. Interestingly, the growth of parasites also accelerated senescence of the uninfected RBCs in parasite cultures if cultured under febrile temperature. These data imply that febrile temperature in association with parasitemia, parasite strain and virulence but not pro-inflammatory cytokines induces more expression of PS molecules on pRBCs. These findings contribute to our understanding of the possible factors that are involved in malaria pathogenesis.

541

THE ANGIOPOIETIN-TIE-2 SYSTEM IS ASSOCIATED WITH RETINOPATHY AND MORTALITY IN MALAWIAN CHILDREN WITH SEVERE MALARIA

Andrea L. Conroy[1], Michael Hawkes[1], Simon Glover[2], Karl B. Seydel[3], Terrie Taylor[3], Malcolm Molyneux[4], Kevin C. Kain[1]

[1]University of Toronto, Toronto, ON, Canada, [2]University of Malawi College of Medicine, Blantyre, Malawi, [3]Michigan State University, Lansing, MI, United States, [4]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Severe malaria is associated with microvascular endothelial activation. The angiopoietins (Ang-1 and Ang-2) and their receptor Tie-2 are important regulators of endothelial quiescence. Previous reports have demonstrated increased Ang-2 and decreased Ang-1 in patients with cerebral malaria (CM). We tested plasma samples from febrile Malawian children who had been prospectively recruited with clinically defined CM (n=155) or moderately severe malaria (n=50) for Ang-1, Ang-2 and the soluble form of Tie-2 (sTie-2). Children's pupils were dilated and the fundi were examined with direct and indirect ophthalmoscopy. Malarial retinopathy was defined by the presence of haemorrhage, whitening, or vessel changes and retinal changes were clinically graded by an experienced ophthalmologist. Of the 205 children enrolled in the study, there were 78 children that had none of the signs of retinopathy, and 117 had one or more. There was a significantly lower median Ang-1 level ($p=0.048$) and significantly higher median Ang-2 ($p<0.0001$) and sTie-2 levels ($p<0.0001$) in children with retinopathy compared to those without. Correlations between retinal grading and the angiopoietins demonstrated an inverse correlation between Ang-1 and retinal whitening (Spearman's rho: -0.153, $p<0.05$), whereas Ang-2 and sTie-2 were positively correlated with retinal whitening and vessel changes (Ang-2:0.421, $p<0.01$; sTie-2: 0.411, $p<0.01$). None of the markers was associated with papilloedema. Finally, median Ang-2 and sTie-2 were higher in those with a fatal outcome (n=61) compared to survivors (n=141), $p<0.001$ for both. Receiver operator characteristic (ROC) curves were generated to assess the prognostic accuracy of the markers and Ang-2 and sTie-2 had areas under the ROC (AUROC) curve of 0.73 (95% CI: 0.66-0.81) and 0.71 (0.64-0.79) respectively. The AUROC of venous lactate, a known prognostic marker in malaria, was 0.66 (0.57-0.75), $p<0.001$. These data suggest that the angiopoietin-sTie-2 system could have diagnostic and prognostic value in children with severe malaria.

542

IMPLICATING COMPLEMENT C5 ACTIVATION IN MALARIA-INDUCED FETAL GROWTH RESTRICTION

Andrea L. Conroy[1], Karlee L. Silver[1], Kathleen Zhong[1], Joseph Fletcher[1], Stephen Rogerson[2], Kevin C. Kain[1]

[1]University of Toronto, Toronto, ON, Canada, [2]University of Melbourne, Melbourne, Australia

Placental malaria (PM) is a leading cause of poor fetal outcomes including spontaneous abortion, preterm delivery and low birth weight. Complement component C5a has been shown to interact with *Plasmodium falciparum* bioactive products (glycosylphosphatidylinositol), to induce synergistic release of inflammatory cytokines and chemokines. Using a murine model of experimental PM (BALB/c mice and *Plasmodium berghei* ANKA), we show that malaria-infected mice had elevated serum C5a at 3 days post infection ($p<0.05$) and elevated levels of placental mRNA encoding the C5a receptor (C5aR) at 6 days post-infection ($p=0.003$). To test the hypothesis that C5a was causally implicated in poor fetal outcomes, we compared pregnancy outcomes of malaria-infected C5aR deficient mice versus wild type (WT). C5aR deficiency resulted in increased fetal viability ($p=0.021$) compared to WT mice and viable fetuses from C5aR-deficient mice experienced less growth restriction ($p<0.001$). Further, C5aR deficiency was associated with improved regulation of angiogenesis. These observations were extended to a population of malaria-exposed women in Malawi. Plasma samples were collected in a case-control design from pregnant women at delivery (n=495). In a univariate analysis, median levels of placental C5a were elevated in women with PM (n=146) compared to women without PM (n=349), $p<0.0001$. There were also changes in angiogenic factors, with decreased angiopoietin-1 (Ang-1, $p<0.0001$), and increased Ang-2 ($p=0.001$) and sFlt-1 ($p=0.002$). In order to extrapolate causal relationships from these data, structural equation modeling was performed using AMOS v17.0 for SPSS. Our model suggests that parasites in the placenta lead to mononuclear cell infiltration, which in turn drives C5a production, dysregulated placental angiogenesis and culminates in fetal growth restriction. Overall, the model was a good fit with an rmsea 0f 0.033 (95% CI: 0.022-0.043). Together, these data suggest that C5a may be an early mediator of placental malaria pathogenesis and may contribute to angiogenic dysregulation and placental insufficiency.

543

PLASMA URIC ACID LEVELS CORRELATE WITH PARASITE DENSITY, INFLAMMATORY CYTOKINE LEVELS, AND DISEASE SEVERITY IN MALIAN CHILDREN WITH MALARIA

Tatiana M. Lopera-Mesa

National Institutes of Health, Rockville, MD, United States

Several *Plasmodium falciparum* factors have been proposed to elicit excessive host inflammatory responses involved in malaria pathogenesis. One of these is uric acid (UA) which can be generated from hypoxanthine and xanthine accumulated by *P. falciparum*-infected red blood cells (RBCs) during parasite maturation. These purines are released at schizont rupture and are converted to UA in plasma by the human enzyme xanthine oxidase. *In vitro*, UA generated in this manner potently activates PBMCs to produce inflammatory cytokines associated with severe malaria. To explore whether parasite-generated UA drives inflammation *in vivo*, we hypothesized that UA levels (i) are elevated during malaria episodes, (ii) positively correlate with parasite density, and (iii) positively correlate with levels of cytokines and chemokines. To test these hypotheses, we measured the plasma levels of UA and inflammatory mediators (IL-1, IL-6, IL-8, TNF, MCP-1, IFNg, IL-10, IP-10, sTNFR II) in 266 Malian children (aged 6 months to 5 years) presenting with their first episode during the 2008 transmission season. Out of 266 children, 248 had mild malaria and 18 had severe malaria (9 with impaired consciousness). UA levels were significantly elevated in children with malaria compared to 18 healthy children without parasitemia ($P=0.001$). Log transformed UA levels correlated with log parasite density ($r=0.26$, $P<0.0001$) and mean values significantly increased with disease severity (mean ± SEM; 4.7 ± 0.09 mg/dl for mild malaria vs. 5.6 ± 0.28 mg/dl for severe malaria, $P=0.002$). Inflammatory mediators also increased with disease severity. Log UA levels positively correlated with log-transformed levels of TNF ($r=0.27$, $P<0.0001$), IL-6 ($r=0.4$, $P<0.0001$), IL-1 ($r=0.22$, $P=0.006$), IL-10 ($r=0.35$, $P<0.0001$), IL-8 ($r=0.29$, $P<0.0001$), MCP-1 ($r=0.27$, $P<0.0001$), IP-10 ($r=0.21$, $P=0.001$), and sTNFR II ($r=0.3$, $P<0.0001$). These data suggest that parasite-derived UA contributes to the pathogenesis of uncomplicated and severe malaria.

544

RELATIVE ANGIOPOIETIN LEVELS ALTER SUSCEPTIBILITY TO EXPERIMENTAL CEREBRAL MALARIA

Karlee L. Silver[1], Sarah Higgins[2], John G. Hay[3], Susan E. Quaggin[4], W. Conrad Liles[5], Kevin C. Kain[5]

[1]McLaughlin-Rotman Centre for Global Health; University of Toronto, Toronto, ON, Canada, [2]McLaughlin-Rotman Centre for Global Health; Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada, [3]Division of Pulmonary and Critical Care Medicine, New York University School of Medicine, New York, NY, United States, [4]Samuel Lunenfeld Research Institute, Mt. Sinai Hospital, Toronto, ON, Canada, [5]McLaughlin-Rotman Centre for Global Health; Department of Medicine, University of Toronto, Toronto, ON, Canada

Cerebral malaria is characterized by endothelial activation. The angiopoietins are critical regulators of the endothelium. Constitutive interaction of angiopoietin (Ang)-1 with endothelial-expressed Tie-2 maintains the integrity and quiescent nature of mature vascular endothelium. Ang-2 can displace Ang-1 from Tie-2 to activate the endothelium to a pro-inflammatory state primed to respond to cytokines such as TNF. Therefore, the relative level of Ang-2 and Ang-1 is believed to play a pivotal role in the regulation of endothelial activation and integrity. Systemic Ang-2 and Ang-1 levels have been shown to be informative biomarkers of malarial disease severity. Due to events indicative of endothelial activation and loss of blood-brain barrier associated with severe malaria, we hypothesized that malaria-induced alteration of Ang-2 and Ang-1 levels plays an important role in the pathogenesis of cerebral malaria. We examined serum and brain Ang levels of inbred mouse strains infected with *Plasmodium berghei* ANKA (PbA): Serum Ang-1 levels of strains susceptible to experimental cerebral malaria (129Sv/J, C57BL/6J and B10.D2/nSnJ) dropped prior to onset of neurological symptoms, and earlier after PbA infection than in resistant strains (B10.D2/oSnJ, AKRJ). Whole brain Ang-2 and the Ang-2/Ang-1 ratio were elevated in infected susceptible B10.D2/nSnJ mice as compared to congenic resistant B10.D2/oSnJ at day 6 post PbA infection. Increased brain Ang-2 was also observed by immunohistochemical staining of brain sections from infected versus uninfected control mice. To show that relative Ang-2/Ang-1 levels were able to determine susceptibility to experimental cerebral malaria, we used an adenovirus gene-delivery system to over-express Ang-1 in susceptible (C57BL/6J) mice and a conditional knockout system to delete Ang-1 in resistant (BALB/c) mice. Reversing the strain susceptibility to experimental cerebral malaria following PbA infection by altering the relative Ang-2/Ang-1 levels supports a pathophysiologic role for these proteins.

545

CYCLOOXYGENASE (COX)-2 PROMOTER HAPLOTYPES ARE ASSOCIATED WITH PROTECTION AGAINST PEDIATRIC SEVERE MALARIAL ANEMIA

Samuel B. Anyona[1], Evans Raballah[1], Prakasha Kempaiah[2], Collins O. Ouma[1], Tom Were[1], Gregory C. Davenport[2], John M. Vulule[3], John M. Vulule[3], James B. Hittner[4], Charity W. Gichuki[5], John M. Ong'echa[1], Douglas J. Perkins[2]

[1]University of New Mexico/KEMRI, Kisumu, Kenya, [2]University of New Mexico, Albuquerque, NM, United States, [3]KEMRI, Kisumu, Kenya, [4]College of Charleston, Charleston, SC, United States, [5]Kenyatta University, Nairobi, Kenya

Inducible cyclooxygenase (COX; prostaglandin-endoperoxide H synthase)-2 is up-regulated by pro-inflammatory mediators and generates the production of elevated levels of prostaglandins (PGs) as part of the host-immune response to infections. We have previously shown that COX-2 transcripts and protein in peripheral blood mononuclear cells, and circulating PGs, are suppressed in children with severe malaria. Although previous studies demonstrated that variation in COX-2 conditions the clinical outcomes in several autoimmune and inflammatory diseases, no studies to date have reported the association between COX-2 genetic variation and susceptibility to severe malarial anemia (SMA). As such, the association between COX-2 -608A/G and -765G/C promoter variants and susceptibility to SMA was investigated among parasitemic children (age: 3-36 months; n=551) with acute malaria presenting at Siaya District Hospital, western Kenya. Demographic, clinical and laboratory measures were determined and children stratified, based on hemoglobin (Hb), into non-SMA (Hb>6.0g/dL; n=316] and SMA (Hb<6.0g/dL; n=235). Genotyping was performed using TaqMan 5′ allele discrimination and PCR-RFLP methods. Proportions of -608 and -765 genotypes were comparable between non-SMA and SMA groups (*P*=0.791 and *P*=0.624, respectively). Similarly, frequencies of haplotype constructs failed to show differences between the groups [-608A/-765C (AC; *P*=0.987), AG (*P*=0.143), and GG (*P*=0.846), respectively]. However, prevalence of the GC haplotype was significantly lower in children with SMA relative to the non-SMA group (*P*=0.016). Multivariate logistic regression analyses, controlling for co-variates, revealed that carriers of GC haplotype had a 78% reduced risk of developing SMA (OR=0.22, 95% CI-0.060-0.782; *P*=0.020). These results suggest that variation at -608 and -765 in the COX-2 promoter may play an important role in conditioning susceptibility to SMA in children resident in *Plasmodium falciparum* endemic areas.

546

ASSOCIATION OF FUNCTIONAL RANTES PROMOTER AND INTRONIC HAPLOTYPES WITH SEVERE MALARIAL ANAEMIA AND MORTALITY IN KENYAN CHILDREN WITH *PLASMODIUM FALCIPARUM* MALARIA

Tom Were[1], Gregory C. Davenport[2], Collins O. Ouma[1], Samuel B. Anyona[1], Prakasha Kempaiah[2], Evans O. Anyona[1], James B. Hittner[3], John M. Vulule[4], John M. Ong'echa[1], Douglas J. Perkins[2]

[1]University of New Mexico/KEMRI, Kisumu, Kenya, [2]University of New Mexico, Albuquerque, NM, United States, [3]College of Charleston, Charleston, SC, United States, [4]KEMRI, Kisumu, Kenya

Regulated on activation normal T-cell expressed and secreted (RANTES, CCL-5) is an important immunoregulatory chemokine that forms part of the intercellular networks that regulate hematopoiesis. We previously showed that suppression of RANTES is associated with severe malarial anemia (SMA, Hb<6.0g/dL) and suppression of erythropoiesis in African children with malaria. Previous studies also demonstrated that genetic variation in RANTES regulates outcomes of inflammatory, auto-immune and infectious diseases, and plasma RANTES levels. However, the role of RANTES gene variation in conditioning SMA, appropriate erythropoiesis (reticulocyte production index, RPI>3.0), mortality and RANTES production in children with malaria is unknown. Associations of RANTES intronic (A+307G, rs2280789) and promoter (G-403A, rs2107538 and A-4120T, rs16971624) haplotypes with SMA, erythropoiesis, three-month post-enrolment mortality, and circulating RANTES were therefore investigated in children (*n*=535) with malaria from western Kenya. Prevalence of the AGT haplotype was 7.1% in the SMA and 13.2% in the non-SMA (Hb>6.0g/dL) groups (*P*=0.024). Multivariate regression modeling controling for covariates showed that haplotype AGT was associated with reduced risk of SMA (OR, 0.501; 95%CI, 0.269-0.934; P=0.030) and appropriate erythropoiesis (OR, 2.247; 95%CI, 1.100-4.591; P=0.026), while haplotype AAA predicted reduced risk of three-month post-enrolment mortality (OR, 0.319; 95%CI, 0.101-1.003; P=0.051). Functional analyses illustrated higher circulating RANTES levels [ng/mL, median (IQR)] in AGT [18.3 (68.3) vs. 11.4 (36.3); P=0.047] and AAA [21.5 (93.1) vs. 11.3 (36.8); P=0.038] haplotype carriers. These results suggest elevated RANTES production, conditioned by genetic variation, is associated with enhanced erythropoiesis, protection against SMA, and reduced malaria-associated mortality.

547

EVALUATION OF PUTATIVE IMMUNOGENIC PROTEINS FROM *VIVAX* MALARIA BLOOD STAGE BY HIGH-THROUGHPUT SCREENING ASSAYS

Eun-Taek Han[1], Jun-Hu Chen[1], Jae-Wan Jung[2], Yue Wang[1], Kwon-Soo Ha[2], Takafumi Tsuboi[3]

[1]Department of Parasitology, School of Medicine Kangwon National University, Chuncheon, Gangwon-do, Republic of Korea, [2]Department of Molecular and Cellular Biochemistry, School of Medicine Kangwon National University, Chuncheon, Gangwon-do, Republic of Korea, [3]Cell-free Science Technology and Research Center and Venture Business Laboratory, Ehime University, Matsuyama, Ehime, Japan

Completed genome sequences and stage-specific transcriptome of the intraerythrocytic developmental cycle of *Plasmodium vivax* offers chances for discovery of new malaria vaccine candidates using innovative screening approaches. Herein, a panel of putative immunogenic antigens from *P. vivax* blood stage was selected using data mining by comparative genomics. Total of 94 ORFs were high-throughput cloned to wheat germ cell-free expression vector (pEU) from 99 PCR products (94.9%) by In-Fusion cloning method. Ninety-five percent (95%, 89/94) genes of *P. vivax* were expressed by wheat germ cell-free system. The putative immunogenic proteins screened with *P. vivax* infected patient sera by protein arrays, a total of 18 (19.1%, 18/94) highly immunoreactive proteins were identified, including 3 of well-characterized vivax vaccine candidates (AMA1, MSP1-42 and MSP1-19), 2 GPI-anchored proteins (MSP8 and MSP10) and MSP3β. Other 12 ORFs have not been previously described as immunologically reactive. These novel immunogenic proteins of vivax malaria blood stage will be further studied as potential vaccine candidates. The results indicates that the In-Fusion cloning method combined with wheat germ cell-free system and protein arrays technology can be used to perform high-throughput screening assays to determine immunogenicity of candidate antigens from the *P. vivax* genome.

548

THE ROLE OF *PLASMODIUM* PARASITES DERIVED MIFS DURING MALARIA INFECTION

Dingding Shao[1], Cong Han[1], Yahui Lin[1], Guangliang Shan[1], Zaixing Zhang[2], Xiaodong Sun[2], Zhensheng Wang[1], Chunyan Wei[1], Yan Deng[2], Lianhui Zhang[1], Lingyi Bu[1], Heng Wang[1]

[1]Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and School of Basic Medicine, Peking Union Medical College, Beijing, China, [2]Yunnan Institute of Parasitic Diseases, Puer, Yunnan Province, China

It has been hypothesized that parasites can modulate the host immune response to benefit their survival by their own molecules,

such as macrophage migration inhibitory factor (MIF). Recently, several *Plasmodium* parasites derived MIFs (*P*MIFs) have been identified to function on host immune cells *in vitro* and furthermore, our group had reported the crystal structure and function comparison between *P*MIF and its host MIF, and confirmed that, 1) as a tautomerase, *P*MIF had a distinct substrate binding pattern and obviously lower enzymatic activity than the host MIF; and 2) both the parasite and host derived MIFs showed identical activities on host cell respectively, however, the combination activities of *P*MIF and host MIF on immune cells was complex. These results suggest the potential regulatory effect of *P*MIF during malaria infection. In this report, by using the monoclonal antibodies specifically against *P. falciparum* MIF (PfMIF) or *P. vivax* MIF (PvMIF), we investigated the correlation of these two *P*MIFs with the course of malaria infection. Data from the epidemiologic studies of the two *P*MIFs shown that the concentrations of the two molecules in the peripheral blood of malaria patients were positively correlated with the level of parasitemia, TNF-α, IL-10 and MCP-1, but not correlated with TGF-β1 and IL-12. Moreover, multiple stepwise regression analysis also showed that parasitemia, IL-10,and HuMIF expression are significant predictors of *P*MIFs production. In addition, by tracing these two *P*MIFs levels during anti-malaria drug treatment, we found decrease of *P*MIFs level following the decrease of parasitemia in most of the patient samples. Our data for the first time shows that the circulating level of *P*MIF is a reflection with *in vivo* malaria parasite density and disease severity and is helpful for further understanding the role of *P*MIF during malaria infection.

549

PLASMODIUM FALCIPARUM ERYTHROCYTE MEMBRANE PROTEIN 1 EXPRESSION IN ISOLATES FROM CHILDREN WITH SEVERE MALARIA

Pamela A. Magistrado[1], Anja T. Jensen[1], Louise Turner[1], Thomas Lavstsen[1], Louise Joergensen[1], Davis John[2], John P. Lusingu[3], Martha Lemnge[3], Raimos Olomi[2], Thor G. Theander[1]

[1]Institute of International Health, Immunology and Microbiology, Copenhagen, Denmark, [2]Kilimanjaro Christian Medical Centre, Moshi, United Republic of Tanzania, [3]National Institute for Medical Research, Tanga, United Republic of Tanzania

Plasmodium falciparum malaria remains one of the world's leading causes of human suffering and poverty. Most deaths are caused by specific severe malaria syndromes such as cerebral malaria, pregnancy-associated malaria, and severe normocytic *P. falciparum*-related anaemia. In areas of stable transmission of *P. falciparum* parasites, mortality and severe morbidity from malaria is restricted to the first 5-10 years of life, as protective immunity is gradually acquired. It is well-established that protective immunity acquired in response to repeated infections is mediated by IgG, and that a principal target of this IgG is parasite-encoded, clonally variant surface antigens (VSA), exposed on the surface of infected erythrocytes. The best-characterized VSA are the family of high-molecular weight (200-400 kDa) proteins called *P. falciparum* erythrocyte membrane protein 1 (PfEMP1). PfEMP1 based vaccines are attractive because these molecules probably are the targets of natural acquired immunity. They are problematic because they are large and diverse. Establishing the parts of the PfEMP1 molecule which are targets for protective antibodies is a key to vaccine development. In another study, PfEMP1 transcription in isolates from Tanzanian children with malaria was analyzed. It was found that severe malaria syndromes such as cerebral malaria, severe anaemia and hyperparasitemia are associated with certain PfEMP1 variants such as VAR4, VAR5, VAR6 and VAR8 while asymptomatic infections are not. In the current study, specific antibodies targeting such PfEMP1 variants were produced and tested in flow cytometry for reactivity with *P. falciparum* isolates taken directly from Tanzanian children with severe malaria. To date, 32% of the 28 isolates tested reacted with VAR4 antibodies. Flow cytometry analyses using PfEMP1 variant specific antibodies and fresh clinical isolates provide clues on PfEMP1 expression in the field. This knowledge aids in the development of a morbidity-reducing malaria vaccine for children in Africa.

550

PLASMODIUM FALCIPARUM GAMETOCYTE CARRIAGE IS ASSOCIATED WITH SUBSEQUENT *P. VIVAX* RELAPSE AFTER TREATMENT

Jessica T. Lin[1], Delia B. Bethell[1], Stuart D. Tyner[1], David L. Saunders[1], Phisit Khemawoot[1], Sabaithip Sriwichai[1], Kurt Schaecher[1], Duong Socheat[2], Steven R. Meshnick[3], Youry Se[1], Chanthap Lon[1], Mark M. Fukuda[1]

[1]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [2]National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia, [3]Gillings School of Public Health, University of North Carolina, Chapel Hill, NC, United States

Up to one-third of patients in Southeast Asia develop *Plasmodium vivax* relapse shortly after treatment of what appears to be a *P. falciparum* (Pf) mono-infection. These patients are thought to harbor cryptic vivax infection not apparent at presentation. A previous retrospective analysis of an artesunate monotherapy trial completed in western Cambodia in 2006-7 showed for the first time that those with *P. falciparum* gametocytes on admission were more likely to develop relapse with *P. vivax* upon follow up. Now results from a larger follow on trial conducted in 2008-9 at the same site further confirms this association. In total, over the two trials, 244 patients with uncomplicated *P. falciparum* malaria received short-acting antimalarials in the form of artesunate monotherapy or quinine/tetracycline for 7 days. 18% of these (44/244) had Pf gametocytes on a peripheral blood smear at presentation. Of those, 55% went on to develop *P. vivax* infection during the 28 or 42 day follow up period, as opposed to 17% of those who were not gametocytemic at presentation. Thus, the presence of Pf gametocytes on an initial blood smear was associated with a 3 fold greater risk of *P. vivax* relapse (RR=3.2, 95% CI 2.1-4.8, $p<0.0001$). This difference could not be explained by duration of illness prior to presentation, initial asexual parasitemia, or history of previous malaria episodes. Patients with a history of malaria in the previous month were excluded. PCR confirmed that a very low proportion of subjects had Pv detectable at baseline, indicating that *P. vivax* parasites resided in the liver at the time of presentation in the majority of patients who relapsed. These data indicate that in areas with substantial rates of mixed *P. falciparum*/*P. vivax* infection, gametocytes seen at presentation may be a potential marker for liver-stage P. *vivax* infection. We hypothesize that the presence of a second competing malaria species may boost *falciparum* gametocytogenesis. If this is true, patients who harbor mixed infection may contribute disproportionately to ongoing malaria transmission.

551

THE GENETIC COMPOSITION OF MULTIPLE-CLONE MALARIA INFECTIONS

Standwell Nkhoma, Shalini Nair, Tim J. Anderson

Southwest Foundation for Biomedical Research, San Antonio, TX, United States

*Plasmodium falciparu*m infections containing multiple clones are common in endemic regions, but the genetic composition of such infections is poorly understood. If these result from superinfection (i.e. sporozoite innoculation from multiple mosquitoes), we would expect component clones to be predominantly unrelated. However, if clones come from a single mosquito inoculation we would expect parasites to be related. To test these predictions, we isolated individual clones from Malawian mixed infections by limiting dilution and genotyped these using 384 SNPs distributed across the genome. We found up to 8 clones per host. There were examples of both related and unrelated parasites within infections, suggesting that both processes occur in nature. The most striking feature of these data was the discovery of parasite clones that are extremely closely related (sharing identity at >90% SNPs genotyped). The differences observed were found in blocks suggesting that the divergent genome regions result from a recombinational process. We suggest two

processes that may be responsible for these observations. First, if multiple clone infections are serially transmitted from one patient to another, then repeated inbreeding will progressively diminish variation among clones, as occurs during generation of recombinant inbred lines in laboratory model organisms such as mice.Alternatively, these divergent blocks could be generated by a novel genetic mechanism such as mitotic recombination, although this is not currently suspected to occur in *Plasmodium*. In conclusion, these data suggest that a simple superinfection model cannot explain the complex relatedness structure observed within multiple clone infections. These results (1) identify a valuable new resource for genetic mapping, (2) refine our understanding of *Plasmodium* population structure and genetics, and (3) have important implications for our understanding of malaria traits such as virulence and sex ratio where kinship is critical.

552

GENETIC DIVERSITY OF THE MEROZOITE SURFACE PROTEINS 8 (MSP-8) AND 10 (MSP-10) IN *PLASMODIUM* SPP

M. Andreina Pacheco, Alamelu D. Elango, Abir A. Rahman, Ananias A. Escalante

Center for Evolutionary Medicine and Informatics, Arizona State University, Tempe, AZ, United States

Assessing how natural selection, negative or positive, operates on genes with low polymorphism is challenging. In *Plasmodium*, the merozoite surface proteins (MSP-1 to MSP-10) are expressed on the merozoite surface. Given their role in the invasion of the red blood cell (RBC), several of them are considered promising vaccine candidates. Among all MSPs identified to date, MSP-1, MSP-8 and MSP-10 have two epidermal growth factor-like domains (EGF) at the C-terminal, which have been proposed to act as ligands during the invasion of RBCs. Those domains are highly antigenic, making them immunogenic and functionally conserved among the different *Plasmodium* species. We investigated the genetic diversity of orthologous genes encoding the Merozoite Surface Protein 8 (MSP-8) and 10 (MSP-10). We applied evolutionary genetic methods to study the polymorphism in MSP-8 and MSP-10 from *Plasmodium falciparum* and *P. vivax*, the two parasites responsible for most human malaria morbidity and mortality. In addition, we studied MSP-8 and MSP-10 orthologous from closely related malarial species found in non-human primates. Overall, genes encoding MSP-8 and MSP-10 are highly conserved in all the *Plasmodium* spp. included in this investigation. Both, MSP-8 and MSP-10, have low polymorphism in *P. falciparum* and *P. vivax* in comparison with the orthologs from other *Plasmodium* species. We found that observed polymorphism in MSP-8 and MSP-10 in *P. vivax* and MSP-8 *P. falciparum* appears to be neutral. There is limited evidence suggesting that MSP-10 in *P. falciparum* could be under positive selection. Yet, we found evidence that the orthologous genes in non-human primate parasites (*P. cynomolgi*, *P. inui*, and *P. knowlesi*) are under purifying (negative) selection. We discuss how selective pressures may differ among orthologous genes in closely related malarial parasites species. It is important to consider the effect of negative selection while studying genes encoding proteins with low polymorphism using comparative approaches.

553

MALARIA MICROSCOPY COMPETENCY IN LIBERIA

Luis Benavente[1], Henry Kohar[2], Joel Jones[2], Hannan K. Bestman[3], Emmanuel Ouma Yamo[4], Kassahun Belay[5], Sean Fennell[1]

[1]Medical Care Development Inc., Silver Spring, MD, United States, [2]National Malaria Control Program, Monrovia, Liberia, [3]Medical Care Development Inc., Monrovia, Liberia, [4]African Medical and Research Foundation, Nairobi, Kenya, [5]United States Agency for International Development, Monrovia, Liberia

Background: In many malaria endemic countries, malaria microscopy is the most common method of malaria diagnosis. However, there are many constraints to achieving competence in malaria microscopy. In addition, in Liberia the war has disrupted the educational system and created a major human resource gap. Until recently, NMCP's training emphasis was in strengthening competence using malaria rapid diagnostic tests (RDTs). Although RDTs are sensitive and specific, they are more costly than microscopy and cannot inform about species or parasite density. Therefore a program of refresher training in malaria microscopy was instituted in Liberia. Methodology: In 2009 and 2010, the National Malaria Control Program (NMCP), the National Public Health Reference Laboratory (NPHRL) and the Improving Malaria Diagnostics (IMaD) project conducted two malaria microscopy refresher training courses for a total of 45 laboratory technicians. The courses combined assessment with training and practice in slide reading. Competency was assessed at the beginning and end of each course using slide sets of known composition. Results of slide reading were graded at the end of every day so participants had the opportunity to review failed slides on the following day.Results: Both theoretical knowledge of malaria diagnosis and performance (sensitivity, specificity, species identification and parasite counting) were very low prior to refresher training. After training overall sensitivity was 84%, overall specificity 87%, species identification 26%, and parasite counting 26%. The percentage of technicians attaining a "pass" level was 54% for sensitivity, 63% for specificity, 0% for species identification and 17% for parasite counting. Out of six microscopists attending both refresher training courses, only two dropped in performance level against the standard although they remained within the 95% confidence interval. The distribution of participants in both training courses combined by quintile (Pf ID) was bimodal both in pre-and post training, suggesting that participants were a heterogeneous mix of high and low performers. Conclusions: There is a need to strengthen skills in malaria microscopy in Liberia using regular training courses that combine assessment with training and practice, especially for national and regional supervisors.

554

NEW LAB-ON-A-CHIP FOR FAST DIAGNOSIS OF HUMAN MALARIA SPECIES AND DETECTION OF DRUG RESISTANCE

Joseph Mugasa, Lisa Ranford-Cartwright

Glasgow University, Glasgow, United Kingdom

Malaria continues to be a major cause of morbidity and mortality worldwide. This has been partly attributed to the resistance of *Plasmodium falciparum* to commonly used antimalarials. Rapid and accurate diagnosis of *Plasmodium* spp is essential for the rational treatment of malaria. Microscopy remains the gold standard tool for malaria diagnosis, but needs skilled manpower, and is tedious and time- consuming. Currently, there are no tools for diagnosis of drug resistance in patients other than relying on reports of failure of curative treatments. Available molecular methods are laborious and time-consuming preventing timely and appropriate decisions on clinical management. Here we describe a collaborative project for the development of a new Lab-on-a-Chip (LoC) based platform (In-check™) for molecular diagnosis of malaria species and drug resistant variants. The In-Check™ Platform is an integrated system combining a fast PCR and microarray based diagnostic test using a single Lab-on-a-Chip. Detection of the five human malaria-causing *Plasmodium* species (*P. falciparum*, *P. vivax*, P. ovale, *P. knowlesi* and P. malariae) is performed using PCR based on 18S rRNA gene followed hybridisation with species-specific probe on the microarray. For detection of drug resistant parasites, polymorphic regions of the genes PfCRT, PfDHFR, PfDHPS and PfCytb that confer resistance to Chloroquine, Sulphadoxine -Pyrimethamine and Malarone are analysed by PCR and hybridisation to specific probes on the microarray. The sensitivity and specificity of the In-Check™ Platform has been assessed using parasite samples at different parasitaemia and samples for which the species present has been determined by other methods. The performance of the In-Check™ Platform relative to slide diagnosis and standard species-specific PCR will be presented. The LoC offers simultaneous diagnosis of the infecting malaria species, together with a prediction of the likely response to commonly used antimalarials for *P. falciparum*. The process takes less than an hour, a considerably shorter period than the current molecular diagnostic tests for malaria.

555

COMBINED RNA AND DNA RT-QPCR ASSAY FOR USE IN MALARIA DIAGNOSIS AND INTERVENTION TRIALS

Edwin Kamau[1], Nancy Nyakoe[2], Linda Muringo[2], Christian F. Ockenhouse[1], John Waitumbi[2]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Walter Reed Project, Kenya Medical Research Institute, Kisumu, Kenya

Microscopy is the gold standard for detection and quantification of malaria asexual parasitemia. Unfortunately, a number of factors mitigate utility of malaria microscopy i.e; the method is poorly reproducible and even then, requires considerable expertise for correct diagnosis and quantification. As such, nucleic acids and antigen based assays are increasingly being used to resolve problems of malaria diagnosis. In a series of studies, we have developed a quantitative real time reverse transcription PCR (RT-qPCR) that is based on amplification of total nucleic acids (RNA and DNA) from 18s rRNA genes for genus *Plasmodium* and the four species of malaria: *Plasmodium falciparum, ovale, malariae and vivax*. For *P. falciparum*, total nucleic acid was extracted using Qiagen Kit from whole-blood samples spiked with cultured, washed, ring-stage-infected red blood cells (iRBCs). The assay has a dynamic range of 0.125-750 iRBCs/µL with Ct values of 37.6 and 21.02 for the lowest and highest parasitemia respectively. By diluting patient total nucleic acid to provide Ct measurements within the dynamic range, *P. falciparum* parasitemia of > 106/µL can be quantified, thus allowing identification of parasite burden within a very broad range. Importantly, the combined nucleic acids RT-qPCR have more than log fold sensitivity over DNA only. We conclude that the combined nucleic acids RT-qPCR is a suitable adjunct to microscopy and could benefit malaria diagnosis and intervention trials.

556

TRENDS IN MALARIA DIAGNOSIS: COMBINING THE USE OF TELEDIAGNOSIS, MICROSCOPY AND PCR IN THE IDENTIFICATION OF *PLASMODIUM* SPP

Blaine A. Mathison, Stephanie Johnston, Maniphet Xayavong, Paul Arguin, Alexandre J. da Silva

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

Microscopic examination of blood smears continues to be the gold standard for laboratory diagnosis of malaria. PCR can also be used for confirmatory testing, especially in cases where an accurate identification cannot be made by microscopy, e.g. low parasitemia, poor preparation or staining, or when a dual infection is suspected. Since 1998, the CDC's DPDx Project has implemented telediagnosis as an effective means of parasite identification, including malaria. Telediagnosis is inexpensive and allows diagnosis to be made in minutes to hours; a good diagnostic alternative in laboratories with adequate infrastructure. Here, we evaluate the results of four years of telediagnosis submissions for malaria, from October 1, 2005 to September 30, 2009 (CDC Fiscal Years 2006-2009). During this time period, the DPDx Team received 1,192 telediagnosis inquiries, 423 of which (35.5%) were for malaria diagnosis. Of the 423 cases, 298 (70.4%) were confidently identified to the species level, identified as *Babesia*, or reported as negative by telediagnosis. However, in 125 (29.6%) of these cases, a definitive identification could not be made by images alone and follow-up material was requested. Requested follow-up material (slides and/or EDTA blood) was received for 79 (63.2%) of the cases. In 36 of the 79 (45.6%) cases where follow-up material was received, the species-level identification was made by microscopy, and in 5 of these 36 (13.9%), PCR further confirmed the microscopy. In 8 of the 79 (10.1%) cases where follow-up material was received, only EDTA blood was received and only PCR was performed. In 33 of these 79 (41.8%) cases, the follow-up examination of smears could not further identify the *Plasmodium* sp. present. PCR was successfully used in 23 of these 33 (69.7%) cases where EDTA blood was also received. Two of the 79 (2.5%) cases where follow-up material was received turned out to be positive for *Babesia* sp. Our data show that telediagnosis is an effective tool for rapid diagnosis of malaria, or to screen clinical specimens for further testing, to better improve patient management.

557

DEVELOPMENT OF A GOLD NANOPARTICLE BASED DIAGNOSTIC ASSAY FOR *PLASMODIUM FALCIPARUM*

Gloria E. Oramasionwu[1], Michael S. Cordray[2], Rebecca R. Richards-Kortum[2]

[1]Baylor College of Medicine, Rice University, Houston, TX, United States, [2]Rice University, Houston, TX, United States

Current malaria diagnosis requires experienced microscopists, expensive equipment, or may be insensitive at low parasitemia, thus a sensitive diagnostic test is needed for limited-resource settings where malaria is prevalent. A recently developed, gold nanoparticle aggregation assay has been shown to specifically detect 107 copies of bacterial DNA. The simplicity of this assay, when coupled with amplification of target DNA, could yield specific malaria diagnosis in low resource settings. Loop mediated isothermal amplification (LAMP) amplifies target DNA up to 109 fold with detection of product by the naked eye, though simple visual turbidity assessment has low specificity. Our goal is to integrate gold nanoparticle mediated aggregation and isothermal amplification to develop a simple, sensitive malaria diagnostic assay that improves upon the low specificity encountered with LAMP. We have employed LAMP to amplify the *Plasmodium* 18s rRNA gene in a region unique to *P. falciparum*. Oligonucleotide-functionalized gold nanoparticle probes were designed to specifically hybridize to adjacent sequences on this amplified portion of the *P. falciparum* 18s rRNA gene in order to generate aggregated complexes of gold nanoparticles in the presence of the target DNA. Target DNA and the gold nanoparticle probes were heated and allowed to hybridize. Upon specific hybridization to the target DNA sequence, the color of the sample changes from pink to purple, the light scattered by the gold changes from green to orange and the shift in scattering spectra is measured by a simple total internal reflection (TIR) spectroscopy device. With LAMP, we are able to amplify and detect as few as 102 copies of the 18s rRNA gene in under 1 hour. Without prior amplification, we are able to specifically detect 333 picomolar or 108 copies/µL of ssDNA, equivalent to 108 parasites using serial dilutions of *P. falciparum* oligonucleotide target and TIR imaging. In conclusion, we have developed an assay that specifically detects the equivalent of 108 *P. falciparum* parasites/µL. Optimization of the isothermal amplification process should improve the limit of detection of the integrated assay diagnostic platform by 107 fold, such that it is comparable to the sensitivity of conventional microscopy, yielding a simple, sensitive alternative for malaria diagnosis in resource-limited environments.

558

PERFORMANCE OF THE TWO RAPID DIAGNOSTIC TESTS FOR MALARIA IN PARA STATE, BRAZILIAN AMAZON

Danielle Regina Barbosa[1], Giselle Maria Viana[1], Nathália Nogueira Chamma[1], Roberto A. Franco[2], Marinete M. Póvoa[1]

[1]Instituto Evandro Chagas, Ananindeua, Brazil, [2]Laboratório Central de Saúde Pública do Estado do Pará, Belém, Brazil

Rapid diagnostic tests (RDTs) for malaria can increase the availability of diagnostic methods, especially in remote areas and in epidemic situations. These tests use immunochromatographic methods to detect *Plasmodium*-specific antigens in blood samples and, differently from microscopy, results can be readily available with minimal equipment and technical expertise. Thus, RDT use tends to increase in the coming years, but they usually present limitations to detect malaria infection, especially in low parasitemic infections. Our objectives were to determine sensitivity, specificity and accuracy of SDBioline® and OptiMAL-IT® in detecting *Plasmodium spp.* in an endemic area of Para State. We used light microscopy as the gold standard. Blood samples were collected from individuals with clinical

signals suggestive of malaria in the Tucurui municipality. Thick smears and RDTs were performed. Out of the 90 samples analyzed, 15.5% (14/90) were positive (range of parasitemia: 0.001%-2%) and 84.4% (76/90) negative by microscopy. OptiMAL-IT® detected 12.2% (11/90) positives and 87.8% (79/90) negatives, and SDBioline® 8.9% (08/90) and 91.1% (82/90), respectively. The statistical parameters obtained by OptiMAL-IT® in relation to microscopy were sensitivity of 78.6%, specificity of 100%, and accuracy of 96.67%; by SDBioline® we found sensitivity, specificity, and accuracy of 50.0%, 96.7% and 91.1%, respectively. Kappa agreement index was 86.1% for the OptiMAL-IT® (almost perfect agreement) and 59.0% for SDBioline® (moderate agreement). These results suggest that OptiMAL-IT® can be used with caution in remote areas of the Brazilian Amazon Region and its sensitivity decline in parasitemias below 500 parasites/µL. For SDBioline®, it is advisable other studies to better assess its performance.

559

DEVELOPMENT OF FIELD-USABLE DRY FORMAT ASSAY FOR THE QUALITATIVE DETECTION OF G6PD ENZYME ACTIVITY

Hyeonsuk Kim, Seung Hee Han, Tae-Hee Koo, Jaean Jung, Young Ho Choi

Access Bio Inc., Monmouth Junction, NJ, United States

Glucose-6-Phosphate Dehydrogenase deficiency is the most common human enzyme deficiency affecting 400 million people. It is an X-linked recessive hereditary disease characterized by abnormally low levels of glucose-6-phosphate (G6PD) and is manifested as anemia, with RBCs being prematurely destroyed by oxidative stress. G6PD deficiency is highly prevalent in malaria endemic areas where 300-500 million people are infected annually with malaria. It implies that many patients that are treated for malaria with oxidative anti-malarial drug will suffer from the treatment because of oxidative stress in their G6PD deficient erythrocytes. To prevent this, it is important to screen G6PD deficiency before treatment with oxidant drugs. Most malaria prevalent areas are economically underdeveloped, and have limited healthcare facilities and limited trained medical care staffs. To meet the need in field situations, we have developed a rapid one step dry format assay for the qualitative detection of G6PD enzyme activity. The assay is based on a formazan method using tetrazolium compound which the color turns yellow to purple under reducing condition. Test strip use capillary power to develop visual signal in the window of device. By using two differential strips with two windows in the device, this test kit allows to distinguish the severity of deficiency. Accelerated stability studies showed that test strips were stable for 2 months at 45oC and 3 days at 60oC. The test procedure is very simple. Just add 2 µl of whole blood to the sample well in the device and followed by adding 2 drops of assay buffer in the assay buffer well. There is no need for the pre-lysis of red blood cells with lysing buffer, a step required by most conventional assays. Since the sample volume needed is only 2 µl, it is possible to use capillary blood as well as venous blood. The assay is rapid (<10 min), easy to operate, inexpensive, portable, and has no special storage requirements.

560

EVALUATION OF TWO RAPID DIAGNOSTIC TESTS FOR MALARIA (OPTIMAL-IT® AND PALUTOP+4®) IN AN ENDEMIC AREA OF PARA STATE, BRAZILIAN AMAZON REGION

Giselle Maria Viana, Nathália Nogueira Chamma, Danielle Regina Barbosa, Ediclei Lima do Carmo, José Maria Nascimento, José Mário Peres, Marinete M. Póvoa

Instituto Evandro Chagas, Ananindeua, Brazil

Rapid diagnostic tests (RDTs) are of simple and fast implementation and some tests may differentiate between *Plasmodium falciparum* and non *P. falciparum*. They are especially useful in situations where conventional microscopy is difficult. This study aimed to evaluate the accuracy of OptiMAL-IT® and PALUTOP+4® in detecting human malaria in Para State, Brazil and to analyze their performance against conventional microscopy and nested-polymerase chain reaction (PCR). Blood samples were collected from individuals with clinical signals suggestive of malaria in the Tucurui municipality. Thick smears, RDTs, and PCR tests were performed. Out of 178 samples, 64.6% (115/178) were positive (range of parasitemia: 0.001%-2%) and 35.4% (63/178) negative by microscopy; OptiMAL-IT® detected 47.8% (85/178) positives and 52.3% (93/178) negatives, and PALUTOP+4® 75.8% (135/178) and 24.2% (43/178) respectively. Nested-PCR detected 66.9% (119/178) positive and 33.2% (59/178) negative samples. The statistical parameters obtained by OptiMAL-IT® in relation to microscopy were sensitivity of 73.9%, specificity of 100%, and accuracy of 83.2%; sensitivity, specificity, and accuracy by PALUTOP+4® were 85.2%, 53.8% and 72.3%, respectively. Kappa agreement index for OptiMAL-IT® was 66.7% when compared to microscopy and 62.4% when compared to nested-PCR (substantial agreement); the same index for PALUTOP+4® was 40.5% (moderate agreement) and 32.0% (fair agreement) compared to microscopy and nested-PCR, respectively. Thus, these results suggest that OptiMAL-IT® can be used with caution in areas of difficult access of the Amazon region and PALUTOP+4® needs further investigation in different transmission settings.

561

EPIDEMIOLOGY OF MALARIA DIAGNOSTICS WITH THE INTRODUCTION OF RAPID DIAGNOSTIC TESTS IN AFRICAN REFUGEE CAMPS, 2007-2008

David A. Townes[1], Christopher Blanton[1], Christopher Haskew[2], S. Patrick Kachur[1], Holly Williams[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]United Nations High Commissioner for Refugees, Geneva, Switzerland

In Africa approximately 1.1 million refugees are under the protection of the United Nations High Commissioner for Refugees (UNHCR), with the majority living in malaria-endemic areas. Historically, malaria has been diagnosed clinically or by microscopy. Introduced in African refugee camps in 2008, malaria rapid diagnostic tests (RDTs) offer a portable, rapid, easy to use and potentially cost effective addition to malaria diagnostics. We describe changes in malaria testing and case confirmation in the year following the introduction of RDTs in refugee camps in six African countries. In the UNHCR health information system (HIS), health indicators, including malaria diagnostics, are recorded on a paper form at camp level and electronically at country level for subsequent analysis. Malaria confirmation is by microcopy or RDT. Malaria diagnostics data from Chad, Ethiopia, Kenya, Sudan, Tanzania, and Uganda (2007-2008) were analyzed with SAS version 9.2 and Microsoft Office Excel 2003. In their first year of introduction, over 105,000 RDTs were performed. Malaria testing increased in five of the six countries (median 352%, *range 20-1543%*) with RDTs accounting for the majority of the increase in Chad, Ethiopia and Tanzania. Malaria testing decreased in Kenya. The percentage of malaria cases that were confirmed increased in Chad, Ethiopia and Kenya (median 19%, *range 10-28%*), decreased in Sudan (5%) and Tanzania (14%) and was unchanged in Uganda. During their first year of introduction, the use and impact of RDTs varied widely. The observed differences among countries are likely due, in part, to inconsistent integration of RDTs into existing guidelines for use of diagnostic tests in suspected malaria, disparities in training of staff on the use of RDTs, and inconsistent availability of both RDTs and microscopy supplies. These results indicate a willingness to use RDTs to supplement existing diagnostics but highlight the need for specific guidelines and training for their integration in these settings to meet 2010 WHO Guidelines for Diagnosis and Treatment of Malaria.

562

A NEWER WAY TO CATCH THE AGE-OLD BUG

Shujaat A. Khan, Deborah Asnis, Elisa Bravo

Flushing Hospital Medical Center, Flushing, NY, United States

Worldwide, an estimated 350-500 million clinical cases and approximately 1 million deaths caused by malaria occur annually, primarily among children aged <5 years living in sub-Saharan Africa (*1*). The majority of the malaria cases diagnosed in US are imported from malaria-endemic regions. A Giemsa-stained blood film is usually the first test for malaria detection but diagnostic accuracy depends on film quality and expertise of laboratory personnel. Effective treatment of malaria requires precise laboratory diagnosis of the four different *Plasmodium* species (*P. falciparum*, *P vivax*, *P ovale* and *P malariae*). A nineteen year old male had recently emigrated from Afghanistan came to the Emergency department with complaints of fever and chills associated with fatigue and generalized myalgia. Physical examination was remarkable for a temperature of 103F and pallor. Initial labs showed hemoglobin of 11.5 g/dL, white blood cell count of 3.4 x 109/L and platelets were 44 x 109/L. Total Bilirubin was 4.1 mg/dL and rest of the blood chemistry was normal. Initial Malaria smear was reported positive for *P Ovale* by both the Hospital Microbiology Lab and State Public Health Laboratory. However, Polymerase chain reaction (PCR) testing of the blood sample later identified the species as *P Vivax*. He was treated with the appropriate dose of Chloroquine followed by primaquine to eliminate latent hypnozoites. The patient responded well to the treatment and was discharged home. In conclusion, successful treatment of Malaria necessitates accurate diagnosis of the offending *Plasmodium* species. Cure of *P vivax* and *P ovale* mandates treatment to eradicate liver hypnozoites where as *P falciparum* infection can result in multiorgan failure requiring parenteral treatment. Microscopy which is quick and cheap can sometimes misidentify the *Plasmodium* species. PCR is a useful complement to microscopy in order to reliably identify the different *Plasmodium* species especially in situations where there is low level of parasitaemia, mixed infections and when there is lack of trained laboratory personnel.

563

QUALITY OF ARTEMISININ-BASED COMBINATION THERAPY PRESCRIPTION AND DISPENSING IN BAMAKO, MALI, WEST AFRICA

Mahamadou Diakite, Eve Sangaré, Samba Diop, Benoit Dembélé, Seidina A. Diakité, Saibou Doumbia, Karim Traore, Drissa Konaté, Sory Ibrahima Diawara, Mory Doumbia, Seydou Doumbia, Sékou Fantamady Traore

University of Bamako, Bamako, Mali

Increasing resistance of malaria parasites to chloroquine has pushed many African countries to adopt artemisinin-based combination therapy (ACT) for the treatment of uncomplicated malaria. Correct use of the ACT strategy is imperative to guarantee the effectiveness of treatment and avoid the spread resistance to ACT. We have conducted this study in order to assess the quality of the prescription and the dispensation of ACTs in randomly selected health centers across Bamako. Our study was a cross-sectional study conducted between April and July of 2008. An interview, via questioner, was administered to patients presenting at the clinic for malaria and to physicians, pharmacists, and other health workers who give prescriptions of antimalarials or work in the pharmacy. In total, 52 prescribers, 72 dispensers and 92 patients were included. Our study has shown that the ACT constituted the primary malaria treatment of choice among prescribers (75%) and dispensers (78.8%). 59.7% of dispensers and 73.1% of prescribers were reported that they were aware of the ACT recommendations by the National Malaria Program (NMCP). The majority of the prescribers (71.15%) and of the dispensers (84.72%) followed the ACT recommendations of the NMCP. However, 57.61% of the prescriptions against malaria did not contain ACT. Many patients (41.30%) did not understand the dosing of the prescribed ACTs which may increase likelihood of emergence of resistance to ACT. Almost all of the prescription containing ACT was a generic drug (97.72%; n = 44). The prices of the ACTs varied between 140 and 3.380 FCFA with an average of 750 FCFA (1 dollar = 500 FCFA). According to prescribers and dispensers, ACT constitutes their first choice (75% of prescribers and 78.8% of the dispensers). However, 57.61% of the prescriptions against malaria did not contain any ACTs. The majority of prescribers (71.15%) and dispensers (84.72%) were favorable to the NMCP's recommendations of malaria treatment in Mali.

564

LOW GAMETOCYTE DENSITIES RESTRICT THE DEVELOPMENT OF *PLASMODIUM FALCIPARUM* WITHIN *ANOPHELES GAMBIAE* WITH IMPLICATIONS FOR THE HUMAN RESERVOIR OF INFECTION AND PARASITE ELIMINATION

Thomas S. Churcher[1], Teun J. Bousema[2], Chris J. Drakeley[2], Caroline Harris[3], Anna Cohuet[4], Isabelle Morlais[5], Dina Vlachou[1], Emma J. Dawes[1], María-Gloria Basáñez[1]

[1]Imperial College London, London, United Kingdom, [2]London School of Tropical Hygiene and Medicine, London, United Kingdom, [3]Institut de Recherche pour le Développement, Montpellier, France, [4]Institut de Recherche en Sciences de la Santé, Bobo-Dioulasso, Burkina Faso, [5]Organisation de Coordination de la lutte contre les Endémies en Afrique Centrale, Yaounde, Cameroon

Density-dependent processes regulating the development of the malaria parasite within the mosquito may influence parasite transmission and could have important implications for the control and the elimination of the parasite. Data from mosquito feeding experiments conducted on naturally found parasite-vector combinations from across Africa were collated to generate a dataset of more than 12,000 mosquitoes which had been fed on blood from a total of 327 different human patients. Gametocytemia was estimated by either microscopy or quantitative nucleic acid sequence-based amplification. Mosquito infectivity was assessed by both the presence of viable oocysts and the number of oocysts identified in infected mosquitoes. A range of mathematical techniques was used to show that the relationship between gametocytemia and oocyst presence and density was best described by a sigmoidal curve, indicating that sporogonic development is restricted at both low and high gametocyte densities. Gametocytemia surveys conducted in Burkina Faso are used to illustrate how these density-dependent regulatory processes will influence the contribution of children to overall transmission. The implications of the results for prospects of malaria elimination are discussed.

565

IMPACT OF INFRASTRUCTURE DEVELOPMENT SUPPORT ON CLINICAL TRIALS CAPACITY DEVELOPMENT IN AFRICA: INDEPTH-NETWORK-MALARIA CLINICAL TRIALS ALLIANCE

Rita Baiden[1], Bernhards Ogutu[1], Peter Smith[2], Fred Binka[1]

[1]INDEPTH Network, Accra, Ghana, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom

The Malaria Clinical Trials Alliance (MCTA), a programme of INDEPTH-Network, was launched in 2006 with two broad objectives: to facilitate the timely development of a network of centres in Africa with the capacity to conduct clinical trials of malaria vaccines and drugs under conditions of Good Clinical Practice (GCP); and to support, strengthen and mentor the centres to facilitate their progression towards self-sustaining research centres. Sixteen research centres or sites in 10 African malaria-endemic countries that were already working with the Malaria Vaccine Initiative or the Medicines for Malaria Venture were selected. Assessment visits based on a standard questionnaire were conducted for all the sites to assess their strengths and requirements for research capacity strengthening, in order to conduct a phase lll malaria vaccine and drug trials. Assessments were made of the needs for infrastructure strengthening and short-term

human capacity development together with the long term sustainability of the centres. Support provided by MCTA included: construction and refurbishment of clinical trial and laboratory facilities, provision of major laboratory and clinical equipment for trials and clinical care, strengthening of the data and financial management systems, GCP and malaria microscopy networking including accreditation and microscopy external quality programmes. Sites were mentored and supported to develop strategic plans for long term sustainability. In 4 years, MCTA strengthened 13 sites to perform internationally acceptable GCP-compliant drug and vaccine trials, including 11 centres that are conducting a very large phase III malaria vaccine trial. The key improvements at the sites, including short and long term impact on the activities of the sites, will be presented. In conclusion, MCTA has demonstrated that clinical research capacity development in Africa is feasible and with modest resources, research centres in Africa can be brought up to GCP compliance standard to conduct research to an internationally acceptable standard.

566

TRENDS IN MALARIA MORBIDITY AMONG HEALTH CARE-SEEKING CHILDREN UNDER AGE FIVE IN MOPTI AND SÉVARÉ, MALI BETWEEN 1998 AND 2006

Seydou Doumbia

Malaria Research and Training Center, University of Bamako, Bamako, Mali

In Mali, malaria is the leading cause of death and the primary cause of outpatient visits for children under five. The twin towns of Mopti and Sévaré have historically had high under-five mortality. We investigated the changing malaria burden in children under five in these two towns for the years 1998-2006, and the likely contribution of previous interventions aimed at reducing malaria. We conducted a retrospective analysis of daily outpatient consultation records from urban community health centers (CSCOMs) located in Mopti and Sévaré for the years 1998-2006, and assessed risk factors for a diagnosis of presumptive malaria using logistic regression and trends in presumptive malaria diagnostic rates using multilevel analysis. Between 1998-2006, presumptive malaria accounted for 33·8% of all recorded consultation diagnoses (10,123/29,915). The monthly presumptive malaria diagnostic rate for children under five decreased by 66% (average of 8 diagnoses/month per 1,000 children in 1998 to 2·7 diagnoses/month in 2006). The multi-level analysis related 37% of this decrease to the distribution of bednet treatment kits initiated in May of 2001. Children of the Fulani (Peuhl) ethnicity had significantly lower odds of a presumptive malaria diagnosis when compared to children of other ethnic groups. In conclusion, presumptive malaria diagnostic rates have decreased between 1998-2006 amongst health-care seeking children under five in Mopti and Sévaré, and a bednet treatment kit intervention conducted in 2001 is likely to have contributed to this decline. Our results corroborate previous findings suggesting that the Fulani ethnicity is protective against malaria. Our findings are useful to encourage dialogue around the urban malaria situation in Mali, particularly in the context of achieving the target of reducing malaria morbidity in children younger than five by 50% by 2011 as compared to year 2000 levels.

567

EPIDEMIOLOGY OF *PLASMODIUM FALCIPARUM, P. VIVAX,* AND ZOONOTIC *P. KNOWLESI* IN SOUTHERN MINDANAO, THE PHILIPPINES

Mary Grace B. Dacuma[1], Rachel Hallett[2], Judeline Dimalibot[3], George Ugaddan[3]

[1]London School of Hygiene and Tropical Medicine and University of the Philippines Los Banos, London, United Kingdom, [2]London School of Hygiene and Tropical Medicine, London, United Kingdom, [3]University of the Philippines Los Baños, Los Baños, Philippines

The Philippines has set the goal to be malaria-free by 2020. However, *Plasmodium falciparum* and *Plasmodium vivax* cases continue to cause significant morbidity in several areas of the country. In Southern Mindanao, transmission 'hot spots' and drug sensitivity, particularly of *P. falciparum*, has not been well studied. Such information is needed to contribute to the overall aim of malaria elimination in this region. We plan to determine whether *P. falciparum* parasites from Southern Mindanao are resistant to chloroquine, sulfadoxine-pyrimethamine, and artemether-lumefantrine using established and proposed molecular markers for drug resistance. We will also determine the transmission intensity of malaria using serological markers of infection and check for the presence of human *P. knowlesi* infection as this has been reported in Palawan in 2008 and in neighbouring Southeast Asian countries. We will conduct a cross-sectional survey in three provinces of Southern Mindanao namely Sarangani and South Cotabato (Region XII), and Tawi-Tawi (Autonomous Region for Muslim Mindanao) from June to July 2010. Consenting participants will answer a questionnaire survey covering personal, demographic and socio-cultural information as well as clinical history of malaria. We will collect a finger prick blood sample from each participant on a rapid diagnostic test strip Falcivax® to check for presence of *P. falciparum* and *P. vivax* malaria, and blood spots on filter paper to be transported to LSHTM for laboratory analyses. *Plasmodium* species presence and *P. falciparum* drug resistance markers will be assessed using molecular methods. We will also use the blood spots to detect and measure antibodies to merozoite surface proteins MSP1 and MSP2, and apical membrane antigen (AMA) using indirect ELISA.

568

MALARIA IN THE CONTEXT OF PREGNANCY: PRELIMINARY RESULTS FROM A QUALITATIVE STUDY IN THE EJISU-JUABEN DISTRICT, CENTRAL GHANA

Arantza Meñaca[1], Nana A. Afrah[2], Lianne Straus[1], Christopher Pell[1], Erin V. Andrew[1], Harry Tagbor[2], Robert Pool[1]

[1]CRESIB, Barcelona, Spain, [2]Department of Community Health, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

This presentation discusses how pregnant women integrate malaria into everyday knowledge and experiences of pregnancy and pregnancy care. The data presented are drawn from a wider anthropological study on the social context of malaria in pregnancy (MiP) in different settings where MiP clinical trials are underway. Data collection included free-listing and sorting exercises, in-depth interviews, group discussions and case studies with pregnant women, their relatives, biomedical and traditional health providers, opinion leaders and other community members. In the Ejisu-Juaben District, pregnancy is regarded as a period when women's bodies are weaker than usual "because of the baby", which is considered to take part of its mother's "blood"/ strength. In this setting, malaria is usually perceived as more frequent and serious in pregnant women than in non-pregnant women. However, knowledge of the risks of MiP, and awareness of malaria infection differ depending on a woman's age, and previous MiP experiences. In order to care for their pregnancies, women attend ANC and use traditional medicines. Nevertheless, biomedicine is the principal choice for the treatment of malaria, and although self-medication is common outside pregnancy, there is a consensus that pregnant women must only take the medicines given to them in the hospital or the clinic. In conclusion, malaria is considered one of the health problems associated with pregnancy, and pregnant women mainly seek treatment for malaria from ANC and maternity wards. Adolescents' lack of accurate knowledge about MiP compounds the vulnerability of pregnant women in this age group.

569

MALARIA AND ANEMIA AT DELIVERY IN HONIARA, SOLOMON ISLANDS

Bridget Appleyard[1], Lyndes Wini[2], Junilyn Pikacha[2], Jeffrey Hii[3], Makiva Tuni[2], Judith Seke[2], James McCarthy[1], Levi Hou[2], Albino Bobogare[2], Anna Maria van Eijk[4]

[1]Queensland Institute of Medical Research, Brisbane, Australia, [2]Ministry of Health and Medical Services, Honiara, Solomon Islands, [3]World Health Organization, Manila, Philippines, [4]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Malaria and anaemia are common problems in pregnancy in developing countries in tropical zones. From May 2009, a cross-sectional survey was undertaken among women who delivered at the National Referral Hospital, Honiara, Solomon Islands to investigate these problems. Information was collected by interview, from obstetric records, and blood was obtained for a maternal smear and haemoglobin assessment. A placental smear was also obtained. By January 2010 1995 women had been enrolled which comprised 57% of all deliveries in the hospital. The prevalence of anemia (Hb< 11 g/dl) was 49.6% and of moderate to severe anemia (Hb< 8 g/dl) 8.2%. Malaria was detected in 5.0% (28/557) of the maternal smears verified to date, mostly *falciparum* (22/28:78.6%); 3 of these women had a temperature > 37.5oC. Malaria was detected in 2.0% (18/866) of the placental smears verified. Risk factors for anaemia included first or second pregnancy (OR 1.2, 95% CI 1.01-1.44, P=0.03); aged under 25 (OR 1.23, 95%CI 1.02-1.48, P=0.03); complicated delivery (OR: 1.9, 95%CI 1.20-3.06, P=0.01); infrequent use of iron and folate supplements (OR 1.28, 95%CI 1.03-1.58, P=0.02); living outside of Honiara (OR 1.27, 95%CI 1.05 to 1.53, P=0.01); and being Polynesian or Micronesian (OR: 1.63;95%CI:1.10-2.37;P=0.01) compared to Melanesian. Use of malaria prevention interventions was common: 44.4% (594/1337) had screened windows, 53.5% (1064/1990) used a bed net during the pregnancy, and 88.5% (1763/1992) reported weekly use of chloroquine prophylaxis. Knowledge of the cause of malaria transmission was high (1631/1907: 85.5%) as was knowledge that malaria is more dangerous for pregnant compared to non-pregnant women (1820/1892: 96.2%). Ante-natal clinic attendance and use of iron and folate supplements was high (1942/1980:98.1%, and 1539/1985:77.5%, respectively). Albendazole was received by 59.4% (1147/1930) of women who attended ante-natal clinic. Final results will be presented at the meeting. In conclusion, while malaria was uncommon at delivery, anaemia was highly prevalent in this population, and requires further study to explore ways to improve this.

570

ANEMIA AND MALARIA IN THE DEMOCRATIC REPUBLIC OF CONGO

Carla C. Hand[1], Steve Taylor[2], Kashamuka Mwandagalirwa[3], Jeremie Muwonga[4], Augustin Okenge[4], Atua Matindii[5], Antoinette K. Tshefu[3], Steven R. Meshnick[1]

[1]University of North Carolina-Chapel Hill, Chapel Hill, NC, United States, [2]Duke University, Durham, NC, United States, [3]Ecole de Sante Publique, Faculte de Medecine, University of Kinshasa, Kinshasa, The Democratic Republic of the Congo, [4]Programme National de Lutte contre le SIDA, Kinshasa, The Democratic Republic of the Congo, [5]Programme National de Lutte contre le Paludisme, Kinshasa, The Democratic Republic of the Congo

Malaria and anemia are both highly prevalent in the Democratic Republic of Congo; however there are many causes of anemia and the importance of malaria remains unclear. We used molecular results from the 2007 Demographic and Health Survey to assess the relationship between malaria and anemia in 4,574 women throughout the country. Blood was collected for on-site hemoglobin testing and stored as dried blood spots, from which genomic DNA was extracted for testing in real-time PCR assays for *Plasmodium falciparum*, *P. malariae*, and *P. ovale*. The prevalence of parasitemia was 28.5% (n=1303). The prevalence of mild (hemoglobin [Hgb] < 11.5 g/dL), moderate (Hgb < 9g/dL), and severe anemia (Hgb<7g/dL) was 33.95%, 14.9% and 1.2% respectively. In bivariate analyses, anemia (defined as Hgb <11 g/dL) was more prevalent in patients living in rural settings compared with urban (32% v. 26%), in poorer compared with wealthier patients (31% v. 26%), in patients with lower body mass indices, in pregnant compared with non-pregnant patients (44% v. 27%), and in patients infected with HIV (47% v. 29% uninfected) or with malaria parasites (32% v. 28% uninfected; all p < 0.01). In a logistic regression model, malaria parasitemia (OR 1.2; 95% C.I. 1.03 - 1.38), HIV infection (OR 2.7; 95% C.I. 1.69 - 4.31), pregnancy (OR 2.3; 95% C.I. 1.91 - 2.66), rural residence (OR 1.4; 95% C.I. 1.13 - 1.62), and low BMI were independently associated with anemia (all p<0.02). Among multi- and mono-species infection, only *P. falciparum* monoinfection was independently associated with anemia (OR 1.2; 95% C.I. 1.05 - 1.42; p < 0.01); combination or monoinfections with P. malariae or P. ovale were not significantly associated with anemia. Independent of other measured correlates, *P. falciparum* is an important contributor to anemia in women the Democratic Republic of Congo.

571

GENES POLYMORPHISMS (CYTOKINE, HBB, G6PD AND TNF) IN A HIGH AND SEASONAL MALARIA TRANSMISSION AREA OF BURKINA FASO

Edith C. Bougouma, Alfred Tiono, Issiaka Soulama, Alphonse Ouedraogo, Amidou Diarra, Amadou T. Konaté, Issa Nébié, Sodiomon B. Sirima

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

Genetic studies showed that genes polymorphism such as Haemoglobin, cytokine, G6PD and ABO are associated with susceptibility to malaria in Africa. These SNP showed powerful selective pressure of malaria (Kwiatkowski and al;, 2000). In this study we aimed to assess genes polymorphism genetic variation of HbS, G6PD, ABO and Cytokine genes according to malaria indicators in children less than five years in a site being characterised for future malaria vaccine trials. The study was carried out in rural villages close to Ouagadougou. We performed a transversal survey during the high malaria transmission season (August). During the survey, we examined 817 children. Blood smears were taken for thick and thin films and a venous sampling was taken for human genetic tests (Cytokine, HbS and ABO). HbAS and HbAC phenotypes were present respectively in 174(19,5%), and 78(8,7%) of 817 subjects. The prevalence of the G6PD genotype was 28,5% for females and 20% for males. IL1A, IL1B were found in 142 (28.7%) and 170 (21.5%) children. IL10 (IL10_232424450), IL10 (hIL-10-1082), IL10 (hIL-10-3533) were found in 395 (50.4%) and 170 (21.5%) subjects. Genotype distribution of children with IL4, IL13 and IL17 was 33.4 %, 28.3% and 48.9% respectively. TNFα376: TNFα308, TNFα238 were respectively 1.5% and 23%. Significant difference was found for Cytokine and TNF (P= 0,001) in term of malaria infection. In conclusion, these results revealed a high frequency of Haemoglobin, cytokines, and G6PD in the study population. These findings suggest that the presence of SNP (Cytokine, G6PD and HBB) could reduce malaria infection. This should be taken into account in the interpretation of malaria trials results.

572

VARIATION IN THE CIRCUMSPOROZOITE PROTEIN OF *PLASMODIUM FALCIPARUM*: IMPLICATIONS FOR MALARIA VACCINE DEVELOPMENT

Kavita Gandhi[1], Mahamadou A. Thera[2], Drissa Coulibaly[2], Karim Traore[2], Ogobara K. Doumbo[2], Shannon Takala-Harrison[1], Christopher V. Plowe[3]

[1]Center for Vaccine Development, University of Maryland, Baltimore, MD, United States, [2]Malaria Research and Training Center, University of Bamako, Bamako, Mali, [3]Howard Hughes Medical Institute, Baltimore, MD, United States

The leading malaria vaccine candidate RTS,S/AS01 is based on immunogenic regions of *Plasmodium falciparum* circumsporozoite protein (CSP) from the 3D7 strain, and has limited efficacy against clinical disease in African children. It is unclear, however, what aspect of the immune response elicited by the vaccine is protective and whether polymorphism in CSP affects efficacy. Better understanding of how diversity in CSP T-cell and B-cell epitopes relates to clinical immunity is needed to evaluate and improve the efficacy of vaccines based on CSP. The goal of this study is to measure diversity in these immunogenic regions and to identify associations between variation in amino acid sequences in CSP and the risk of infection and clinical disease caused by *P. falciparum*. A prospective cohort study was conducted to measure the age-specific incidence of malaria infection and disease in children and young adults living in Bandiagara, Mali. For this study, a subset of 100 children contributed 2,309 samples of finger-stick blood spots collected in asymptomatic monthly surveys and during acute clinical malaria episodes. Amplified T-cell and B-cell regions of the cs gene are being subjected to 454 sequencing, a powerful method for detecting diversity in complex infections. Cox proportional hazards models are being used to determine the effect of sequence variation in individuals' consecutive infections on the time to new infection and new clinical malaria episode. Preliminary analyses revealed that with >500X average coverage (range ~200-1,000X), 454 sequencing of 45 randomly selected samples had a high degree of variation in the T-cell regions Th2R and Th3R in the form of single nucleotide polymorphisms (SNPs). 24 SNPs were found in Th2R and 14 in Th3R, all non-synonymous, resulting in 72 unique Th2R haplotypes and 14 Th3R haplotypes. Only three of 45 samples (7%) are identical to the 3D7 vaccine strain in both epitopes, raising the possibility that parasite genetic diversity may limit efficacy of CSP-based vaccines, if protection is strain specific.

573

IS THE RISK OF *PLASMODIUM FALCIPARUM* MALARIA INCREASING IN VERY YOUNG CHILDREN WHO ARE VFRS? ARE THEIR PARENTS CONSCIENTIOUS OF THIS RISK?

Antonio Soriano Arandes, Olga Calavia Garsaball, Frederic Gómez Bertomeu, Clara Bras Boqueras, Sergi César Díaz, Meritxell Gellida Royo

Hospital Universitari Joan XXIII, Tarragona, Spain

Plasmodium falciparum malaria is a highly endemic disease in most of the sub-Saharan countries. Nigeria has the largest population of Africa and their emigrants to Tarragona (Spain) represent the second African number of inhabitants in this region. It is known that immigrant people returning home to visit friends and relatives (VFRs) are the highest risk traveling population for contracting malaria because they lost their pre-existing acquired immunity against *P. falciparum* and they also assume that they are "immune" for the infection leading to a lower compliance of the anti-malarial prophylaxis. The epidemiological patterns of VFRs are changing and, as a consequence of better socioeconomic and life conditions in the country of residence, more immigrant families are now traveling with their children to the African countries. We describe three pediatric cases of malaria diagnosed at the University Hospital Joan XXIII, Tarragona (Spain), member of the *TropNet Europ* for imported infectious diseases surveillance, in a one-year period 2009-2010. They all were born in Tarragona and diagnosed after traveling with their family as VFRs to Nigeria with a mean stay of 83.3 days. Two children received pre-travel counselling and anti-malarial prophylaxis with mefloquine but none of them achieved a good compliance. The youngest case was unable to receive mefloquine because of his age (4-month old) and the no seeking pre-travel health counseling. Ages ranged from 4 to 17 months old. Day of onset of the fever ranged from 6 to 13 days with a mean of 9.7 days after returning of the travel. In spite of being visited twice at emergency department of the hospital, diagnosis of malaria was delayed in two patients for 7 days. One of them was classified as a complicated malaria (parasitemia of 5%) complying criteria established by the WHO. PCR technique was positive for *P. falciparum* in the three cases. Lowest hemoglobin was 5.13gr/dL and lowest hematocrit was 14.6%. All of them were treated as inpatient cases with intravenous quinine and clindamycin and two also received antibiotic for associated bacterial pneumonia. The purpose of this study is to show these pediatric malarial cases to sensitize the medical professionals working in Tropical Medicine for the increasing importance of very young children travelling as VFRs and having a high risk to contract malaria in their familial origin countries.

574

ACTIVE SURVEILLANCE OF MALARIA IN MILITARY AREAS OF OPERATION (AOS) ALONG NORTHERN THAI-MYANMAR AND THAI-NORTHERN CAMBODIA BORDERS DURING 2004-2009

Narupon Kuttasingkee, Toon Ruang-areerate, Pradith Kaewsatien, Kiatisak Somsri, Khwananong Youngpakool, Bungauang Indontri, Chaiya Chanchu, **Jariyanart Gaywee**

Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

Military areas of operation (AOs) along Northern Thai-Myanmar and Thai-Cambodia borders are known as malaria endemic. Therefore, immune naive troops who are deployed to these areas are at risk for malaria infection. Upon infection, soldiers are evacuated from the AO causing a force reduction. Malaria surveillance is crucial for effective malaria prophylaxis and decreases disease non-battle injury (DNBI). We have conducted a continuous surveillance program to obtain the epidemiological information of malaria in these bordered AOs since 2004. Each fiscal year, area-deployed troops were monthly screened for malaria infection in peripheral blood using rapid test and microscopic confirmation. Additional surveillance data were also collected from the healthcare providers in the area. From 2004 to 2009, malaria infections in army troops deployed to AO along northern Thai-Myanmar border were 13.2%, 5.1%, 9.6%, 5.2%, 4.9% and 12.2%, respectively. Whereas in AO along Thai-northern Cambodia border, 8.2% 4.0% 7.4% 8.6% 4.2% and 21.3% of deployed troops were infected with malaria during the same period. Malaria cases occurred in two peaks every year from October to February and May to July. An interesting point was that the incidence of *P. vivax* increased each year and implied a shift to primary malaria infection. Active surveillance and additional data must be collected and studied to provide understanding and implementation of efficiency protective programs and thus reduction of DNBI.

575

PREVALENCE OF MALARIA INFECTIONS AND RELATED MORBIDITY AMONG SCHOOL CHILDREN IN PARTS OF THE IMO RIVER BASIN, NIGERIA

Ikechukwu N. Dozie[1], Ijeoma J. Dozie[2], Uchechukwu M. Chukwuocha[1]

[1]Federal University of Technology, Owerri , Nigeria, [2]Federal Medical Centre, Owerri, Nigeria

This study was designed to investigate malaria signs and rates of parasiteamia among school children in Ezinihitte Local Government Area in the Imo River Basin, Imo State, Nigeria.Malaria parasite and degree of

anaemia were assessed in 469 selected primary school children, using standard parasitological and haematological mthods of diagnosis. Clinical examination was done to determine spleen size. About 12.8% of the pupils were positive for malaria parasites, 48.6% were anaemic and 11.3% had spleen enlargement. Also 4.9% of the study pupils had all three of the symptom. This study ascertained a significant association between malaria infections, anaemia, and splenomegally and identified the study area as a high risk area for malaria.There is need to enhance malaria control efforts to reduce the level of morbidity among children in the study area so as to make them more effective at school.

576

CONSEQUENCES OF PREGNANCY-ASSOCIATED MALARIA ON FETAL GROWTH IN KOROGWE, TANZANIA

Christentze Schmiegelow[1], Birgitte B. Nielsen[2], Vibeke Rasch[3], Thomas Scheike[4], Mayke Österholt[5], Daniel Minja[6], Pamela Magistrado[1], Stephanie Böstrom[7], Lydia Massawe[8], Martha Lemnge[9], John Lusingu[9], Thor Theander[1]

[1]Center for Medical Parasitology at the Institute for International Health, Immunology and Microbiology, University of Copenhagen and Copenhagen University Hospital, Copenhagen, Denmark, [2]Department of Obstetrics and Gynaecology, Aarhus University Hospital, Skejby, Denmark, [3]Department of Obstetrics and Gynaecology, Odense University Hospital, and Department of International Health at the Institute for International Health, Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark, [4]Department of Biostatistics at the Institute of Public Health University of Copenhagen, Copenhagen, Denmark, [5]Department of Medical Microbiology, Radboud University, Nijmegen, The Netherlands, [6]National Institute for Medical Research, Tanga Medical Research Center, Tanga, United Republic of Tanzania, [7]Department of Immunology at the Wenner-Gren Institute University of Stockholm, Stockholm, Sweden, [8]National Institute for Medical Research Tanga Medical Centre, Tanga, United Republic of Tanzania, [9]National Institute for Medical Research, Tanga Medical Research Centre, Tanga, United Republic of Tanzania

Pregnancy-associated malaria (PAM) has detrimental effects on mother and fetus. PAM causes low birth weight due to intrauterine growth retardation (IUGR), but the time of onset of IUGR is unknown. Recent studies have shown a relation between PAM and development of hypertensive disorders (Pregnancy Induced Hypertension (PIH) and Preeclampsia (PE)), which themselves can affect the fetus. The interrelation between PAM and hypertensive disorders is not thoroughly investigated. Furthermore, a fetal growth chart representative for an East African population is currently not available. The objective of the study is to investigate the effects of PAM on fetal growth and on the development of hypertensive disorders. A longitudinal prospective study of 1000 pregnant women is conducted in Korogwe, North-eastern Tanzania. Using ultrasound investigation, the gestational age is estimated before 24 weeks of gestation. Fetal growth is assessed on at least three consecutive ultrasound investigations, enabling us to diagnose IUGR. In parallel, screening for malaria, PIH and PE is performed during pregnancy and at delivery. PAM is diagnosed using Rapid Diagnostic Tests and placental histology. Using data from offspring of healthy mothers not diagnosed with PAM, PIH or PE a normal Tanzanian cohort is generated and a growth chart is produced. The prevalence and time of onset of IUGR among fetuses carried by mothers suffering from PAM and/or hypertensive disorders is investigated and compared with this normal cohort. Follow-up is ongoing. Preliminary analysis indicates a relative high prevalence of IUGR among women who had parasiteamia during the pregnancy. The onset of malaria induced IUGR occurs earlier in pregnancy among the primigravidae than in the multigravidae. Hence, the effect on birth weight is more pronounced among primigravidae. Furthermore a correlation is seen between PAM and the development of hypertensive disorders..

577

ASYMPTOMATIC *PLASMODIUM FALCIPARUM* INFECTIONS IN NAVRONGO, NORTHERN GHANA: A NEW ANALYSIS METHOD SUGGESTS DIFFERENCES IN CLEARANCE OF INFECTIONS COMPARED TO MALARIA THERAPY DATA

Michael T. Bretscher[1], Wilson Sama[1], Nicolas Maire[1], Nakul Chitnis[1], Seth Owusu-Agyei[2], Tom Smith[1]

[1]Swiss TPH, Basel, Switzerland, [2]Kintampo Health Research Center, Kintampo, Ghana

To understand the likely impact of preventive measures, it is important to know how long untreated infections persist. Longitudinal genotyping data represents the main source of information on the dynamics of natural *Plasmodium falciparum* infections. However, statistical analysis of such data is not straightforward due to ongoing re-infection and the problem of imperfect detection of asymptomatic infections. Specialized analysis methods which simultaneously estimate force of infection, detectability, and duration of infections have therefore been developed.We have now extended these methods to not only measure a mean duration of infection, but rather use various survival distributions to describe clearance of infections. This is an important step in an iterative model-finding process, which ultimately leads to a better understanding of the within-host processes in naturally exposed populations. We have applied our method to msp2 genotyping data from a one-year longitudinal study on asymptomatics in all age groups, conducted in Navrongo, Northern Ghana. The results suggest pronounced differences in the distribution of infection durations compared to malariatherapy data. Part of the infections in the natural population appear to be of relatively short duration, with some infections persisting for a long time. By using age of the human host as a proxy for cumulative exposure, we were able to exclude acquired immunity as possible cause. This indicates that other factors, such as the genetics of human or parasite populations may be responsible for the observed differences between natural infections and malariatherapy data. Ongoing research investigates the robustness of these results with respect to more explicit modeling of additional features of within-host dynamics, such as the decrease of parasite densities over the time course of an individual infection, which lowers the probability of detection.

578

QUANTIFYING THE BURDEN OF PREGNANCY-ASSOCIATED MALARIA IN THE DEMOCRATIC REPUBLIC OF THE CONGO

Steve M. Taylor[1], Jane Messina[2], Carla C. Hand[1], Kashamuka Mwandagalirwa[3], Antoinette K. Tshefu[3], Atua Matindii[4], Jeremie Muwonga[5], Michael Emch[2], Steven R. Meshnick[1]

[1]University of North Carolina Gillings School of Global Public Health, Chapel Hill, NC, United States, [2]University of North Carolina Department of Geography, Chapel Hill, NC, United States, [3]Ecole de Sante Publique, University of Kinshasa, Kinshasa, The Democratic Republic of the Congo, [4]Programme National de Lutte contre le Paludisme, Kinshasa, The Democratic Republic of the Congo, [5]Laboratoire National de Reference SIDA et IST, Kinshasa, The Democratic Republic of the Congo

Pregnancy-associated malaria reliably contributes to poor birth outcomes, but its true prevalence is unknown. We report the first estimate of burden based on a nationally-representative survey. Malaria prevalence was measured by real-time PCR in 4,574 women aged 15-49 years old responding to the 2007 Democratic Republic of the Congo Demographic and Health Survey. 520 (11%) women were pregnant when surveyed, and a further 954 (21%) women had delivered in the previous year; overall, median gravidity was 2 (IQR 2-5). Only 32% of pregnant women possessed a bednet, and 24% of all pregnant women slept under a bednet the previous night. Overall, 1,253 women (27%) were parasitemic with *Plasmodium falciparum*; there was no significant difference in parasite prevalence between pregnant women (31%) and nonpregnant women (27%; p=0.18) or between pregnant women in different

trimesters. Additionally, there was no significant difference in parasite rate between those who slept under bednets the previous night that were treated with insecticides (27%) or untreated (19%) and those who used neither (27%; p=0.32). Gravidity was associated with parasite prevalence, with primigravidae (41%) more frequently infected than secundigravidae (32%) and multigravidae (27%; p=0.02). *P. malariae* and *P. ovale* infected < 2% of women, and there were no significant differences in parasite rates by pregnancy or trimester. An estimated 3 million pregnancies occur every year in the DRC; with at least 31% of pregnant women infected with malaria, over 1 million episodes of pregnancy-associated malaria may occur every year. There is an urgent need for interventions to prevent malaria in pregnancy in the DRC.

579

IMPACT OF SUBSIDIZED ARTEMETHER-LUMEFANTRINE (AL) IN THE RETAIL SECTOR ON COVERAGE OF PROMPT EFFECTIVE TREATMENT OF CHILDREN UNDER FIVE IN KENYA: A CLUSTER RANDOMIZED CONTROLLED TRIAL

Beth B. Kangwana[1], Sarah V. Osano[1], Abdisalan M. Noor[1], Victor A. Alegana[1], Andrew J. Nyandigisi[2], Jayesh Pandit[3], Manya Andrews[4], Mbogo Bunyi[4], Greg Fegan[1], Simon Brooker[1], Robert W. Snow[1], Catherine A. Goodman[1]

[1]Kenya Medical Research Institute Wellcome Trust Research, Nairobi, Kenya, [2]Division of Malaria Control, Ministry of Public Health and Sanitation, Nairobi, Kenya, [3]Pharmacy and Poisons Board, Nairobi, Kenya, [4]Population Services International, Nairobi, Kenya

With a low proportion of children receiving the first line treatment for suspected malaria, a study was implemented to evaluate the impact of providing subsidized AL delivered through trained retail providers, and supported by communications on the coverage of prompt effective anti-malarial treatment in children 3-59 months. We employed a pre-post randomized cluster controlled design with nine control and nine intervention sub-locations, equally distributed across three districts in western Kenya. Three clusters of villages were randomly selected within each sub-location using probability proportional to size sampling, and 42 homesteads were randomly selected per cluster to participate in a household survey on treatment seeking behavior. Data was collected using structured questionnaires and analyzed using a difference in difference approach based on cluster level summaries, comparing control to intervention areas. A total of 2,749 children aged between 3 and 59 months were recruited, of which 2,662 were followed up 12 months later. 29% of children experienced fever within two weeks prior to the interview. At follow up, the percentage of children with fever receiving AL had risen by 17.5% points in the control arm (9.80% (SD:8.27) to 27.30% (SD:15.22) and 45.95% points in the intervention arm (7.74% (SD:5.05) to 53.69% (SD:12.29). The percentage of children receiving AL in the intervention arm at follow up was significantly greater than in the control arm (p=0.0001). No significant differences were observed between arms in where caregivers sought treatment for their child's fever, or in the child's adherence to AL (p>0.05). Subsidizing ACT in the retail sector can significantly increase coverage of prompt and effective treatment of malaria in rural areas. The increase in coverage observed in the control areas probably reflected improved availability of AL in public health facilities, highlighting that ensuring health facility AL stocks is also essential for improving AL access.

580

RECONSTRUCTION OF INDIVIDUAL MULTILOCUS GENOTYPES FROM MIXED *PLASMODIUM* INFECTIONS

Jordan Kemere[1], Paul Chaffee[2], Inna Gerlovina[2], Curt Hansen[2], Bryan Greenhouse[1]

[1]University of California at San Francisco, San Francisco, CA, United States, [2]University of California, Berkeley, Berkeley, CA, United States

Obtaining multilocus genotypes of *Plasmodium* parasites is essential in numerous applications in population genetics, such as tracking drug resistant parasites and understanding transmission. Determining genotypes of individual strains is challenging when multiple strains co-infect a single patient, as it is difficult to know which allele to assign to which strain. As a result, these types of infections, which are common in areas of moderate or high transmission intensity, are often ignored in population genetic analyses requiring multilocus genotypes. We developed a heuristic algorithm based on maximum likelihood to address these challenges for semi-quantitative microsatellite genotypes. The algorithm first estimates the number of unique strains and relative proportion of each, then assigns alleles to each strain. We estimated the number of strains present in a sample as the maximum number of alleles present at any given locus across all loci measured. The proportion of each strain and the allele assignments were then iteratively estimated. For loci at which multiple strains share an allele, we used maximum likelihood to assign alleles at each locus to a particular strain, optionally using a priori information about the population frequency of particular alleles or estimating these iteratively from a large data set. To test the algorithm, we simulated data for 2, 3, and 4 strains present at 10 loci based on empiric microsatellite allele frequencies measured from patient samples in Uganda. Assuming an average quantification error of 1%, the algorithm correctly assigned 99%, 97%, and 93% of alleles for 2, 3, and 4 strains. Assuming an average quantification error of 3%, the algorithm correctly assigned 97%, 91%, and 81% of alleles. Laboratory experiments are in process to determine the accuracy of both the microsatellite quantification and the algorithm using real data from 201 mixtures of 9 laboratory strains. We have developed a novel algorithm which may enable accurate determination of multilocus microsatellite genotypes from mixed *Plasmodium* infections.

581

PRECLINICAL PRIORITIZATION OF BLOOD STAGE MALARIA VACCINE CANDIDATES

Julie Healer[1], Alex Uboldi[1], Ping Cannon[1], Tony Hodder[1], Jack Richards[1], Matt O'Neill[1], Alex Maier[1], Satoru Takeo[2], Thangavelu Arumugam[2], Motomi Torii[2], Takafumi Tsuboi[2], James Beeson[1], Brendan Crabb[3], Alan Cowman[1]

[1]Walter & Eliza Hall Institute of Medical Research, Melbourne, Australia, [2]Ehime University, Matsuyama, Japan, [3]Burnet Institute, Melbourne, Australia

A licensed vaccine for use in humans against *Plasmodium falciparum* malaria is not yet available. Until a vaccine based solely on stimulating immune responses against pre-erythrocytic developmental stages is proven to be clinically effective, there remains a need for discovery of novel candidate antigens of all life cycle stages, which can be further assessed for their potency in inducing efficacious anti-malarial immunity. We have initiated a functional screening approach to prioritizing blood-stage candidates for clinical development. Using a genome-wide approach, a cluster of 11 genes that putatively encode merozoite antigens including the MSP3/MSP6 family of proteins have been identified in a 43 kb region of *P. falciparum* chromosome ten. Population genetics studies have highlighted members of this gene cluster as among the most diverse in the genome and it is believed that immune selection has played a major role in generating this diversity. In support of this, some of these antigens are known to be targets of protective immunity have been subject to investigation as potential vaccine candidates. To assess the functional role of these antigens in merozoite invasion and to determine their potential

utility as vaccine candidates, we have characterized all of these antigens with respect to their cellular localization and interaction with red blood cells, their ability to elicit protective antibody responses and the capacity of parasites lacking these antigens through genetic deletion to invade red blood cells.

582

ANALYSIS OF AN EXTENSIVE INTEGRATED SAFETY DATABASE OF PEDIATRIC PHASE II CLINICAL TRIALS WITH THE RTS,S/AS CANDIDATE MALARIA VACCINE

Johan Vekemans, Yolanda Guerra, Marc Lievens, Sarah Benns, Didier Lapierre, Amanda Leach, Tom Verstraeten

GlaxoSmithKline Biologicals, Rixensart, Belgium

The RTS,S/AS malaria vaccine candidate is currently in Phase III clinical development in Africa. Before progressing to Phase III, safety data from 9 pediatric Phase II trials were pooled. Serious adverse events (SAE) during the whole follow-up period, unsolicited adverse events (AE) within 30 days post vaccination and laboratory safety blood markers were assessed. Relative risks (RR; RTS,S/AS over control) were calculated by Poisson regression models adjusted for study, without adjustment for multiplicity of analyses. P-values were calculated using an exact stratified test conditional on the number of cases. 2981 children under 5 years, including 617 aged <12 weeks at first vaccination, received a total of 8860 doses. The mean follow-up was 23.3 months. RTS,S/AS vaccination was associated with a significantly lower reporting rate of any SAE compared to control (RR 0.77 95%CI: 0.68-0.87;p<0.001) and a 2-fold reduction of all fatal reports (15 RTS,S/AS vs 29 controls; RR 0.49 95%CI: 0.24-0.94;p=0.031). No SAE was reported more frequently after RTS,S/AS. RTS,S/AS vaccination was associated with a reduction in malaria (RR 0.70 95%CI: 0.57-0.85;p=0.003), *P. falciparum* infection (RR 0.70 95%CI: 0.53-0.92;p=0.011), severe malaria (RR 0.61 95%CI: 0.43-0.85;p=0.003) and pneumonia (RR 0.71 95%CI: 0.52-0.97;p=0.032). After excluding malaria-related SAE, RTS,S/AS recipients still recorded significantly fewer SAE (RR 0.81 95%CI: 0.69-0.95; p=0.008). Two cases of RTS,S/AS-related simple febrile convulsions were reported. With respect to AEs, RTS,S/AS recipients recorded more upper respiratory tract infections (27.1 vs 20.6%;p=0.002), dermatitis diaper (0.6 vs 0%;p=0.01) and rash (1.1 vs 0.5%;p=0.032) than controls. *P falciparum* infection (1.1 vs 1.3%;p=0.011) and rhinorrhoea (4.1 vs 5.1%;p=0.048) were reported less frequently. Abnormal laboratory values were infrequent and usually not clinically significant. Analysis of a RTS,S/AS vaccine safety database confirms the favorable safety profile of RTS,S/AS in children and infants, and supports further Phase III assessment.

583

HETEROLOGOUS ADENOVECTORED VACCINE REGIMENS ARE IMMUNOGENIC AND PROTECTIVE IN THE *PLASMODIUM YOELII* MOUSE MALARIA MODEL

Noelle B. Patterson[1], Maureen E. Stefaniak[1], Kalpana Gowda[2], Fouzia Farooq[1], Shannon McGrath[3], Svetlana Konovalova[4], Ping Chen[4], Elena Semenova[4], Richard Grier[4], Joseph T. Bruder[4], Keith Limbach[1], Thomas L. Richie[2]

[1]United States Military Malaria Vaccine Program, Naval Medical Research Center, Henry M. Jackson Foundation for the Advancement of Military Medicine, Silver Spring, MD, United States, [2]United States Military Malaria Vaccine Program, Naval Medical Research Center, Silver Spring, MD, United States, [3]United States Military Malaria Vaccine Program, Walter Reed Army Institute of Research, Silver Spring, MD, United States, [4]GenVec, Inc., Gaithersburg, MD, United States

Adenovector serotype 5-based vaccines encoding two *Plasmodium falciparum* antigens (CSP and AMA1) developed and tested by our program induced sterile protection against sporozoite challenge in Ad5 seronegative humans primed with DNA plasmids encoding CSP and AMA1. However, the efficacy of this prime-boost regimen may not extend to humans with preexisting neutralizing antibody specific to Ad5 (Ad5NAb). To address this concern, we developed adenovectored malaria vaccines designed to circumvent preexisting adenovirus-specific neutralizing antibody, including alternate serotype-based (Ad28 and Ad35) and hexon/fiber-modified adenovectored vaccines. We used the *P. yoelii* circumsporozoite protein (PyCSP) as the model antigen to test this approach. The new Py adenovectors that grew well and robustly expressed PyCSP *in vitro* were evaluated for immunogenicity and protective efficacy. BALB/c mice were immunized with a heterologous two-dose regimen given 6 weeks apart of varying prime-boost combinations, utilizing DNA, Ad5, Ad28, and/or Ad35 vectors. Two weeks post-boost, mice were either utilized for immunogenicity testing (ELISA, intracellular cytokine staining for multifunctional T cells, and ELISpot responses to PyCSP) or challenged with *P. yoelii* sporozoites intravenously and monitored for malaria parasitemia via blood smears. Here we report that alternate serotype malaria vaccines, used in certain heterologous prime-boost combinations, induced malaria-specific T cell and antibody responses comparable to the DNA/Ad regimen. While several alternate serotype malaria vaccines provided protection against malaria, the Ad28/Ad5 and Ad35/Ad5 regimens were the most promising. Specifically, Ad28/Ad5 (protection 36-71%; n=28) and Ad35/Ad5 (43-50%, n=28) elicited better protection than Ad5/Ad5 (14%, n=14) or Ad5 single administration (0%, n=14), respectively. Ad28/Ad5- and Ad35/Ad5-induced protection was comparable to that of DNA/Ad5 (29-43%, n=28). Subsequent experiments will evaluate the ability of the successful regimens to avoid preexisting Ad5NAb.

584

PHASE 1 STUDY OF BSAM2/ALHYDROGEL+CPG 7909 IN MALARIA NAÏVE UNITED STATES ADULTS

Ruth D. Ellis[1], Donna Shafer[2], Yimin Wu[3], Kazutoyo Miura[4], Joan Aebig[3], Kelly Rausch[3], Daming Zhu[3], Laura Martin[3], Michael Fay[5], Carole Long[4], Louis Miller[3], Anna Durbin[2]

[1]NIAID, Rockville, MD, United States, [2]Johns Hopkins Center for Immunization Research, Washington, DC, United States, [3]Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [4]Biostatistics Research Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [5]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

A Phase 1 dose escalating study was conducted in malaria naïve adults to assess the safety, reactogenicity, and immunogenicity of the blood stage malaria vaccine BSAM2/Alhydrogel + CPG 7909. BSAM2 is a combination of the FVO and 3D7 alleles of AMA1 and $MSP1_{42}$, with equal amounts of each of the four proteins mixed, bound to Alhydrogel, and administered with the novel adjuvant CPG 7909. 30 volunteers were enrolled in two dose cohorts, with 15 volunteers receiving up to three doses of 40 µg protein at Days 0, 56, and 180, and 15 volunteers receiving up to three doses of 160 µg protein on the same schedule. Most related adverse events were mild or moderate, but 4 volunteers experienced severe systemic reactions and two were withdrawn from vaccinations due to adverse events. Antibody responses were not significantly different in the high dose versus low dose groups, and did not further increase after third vaccination. *In vitro* growth inhibition was demonstrated and was closely correlated with anti-AMA1 antibody responses.

585

COMPARISON OF *PLASMODIUM BERGHEI* CHALLENGE MODEL FOR THE EVALUATION OF PRE-ERYTHROCYTIC MALARIA VACCINES AND THEIR EFFECT ON PERCEIVED VACCINE EFFICACY

Elke S. Bergmann-Leitner[1], Wolfgang W. Leitner[2], Evelina Angov[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]National Institute of Allergy and Infectious Diseases-National Institutes of Health, Bethesda, MD, United States

The immunological mechanisms responsible for protection against malaria infection vary between different *Plasmodium* species, host species and the developmental stage of the parasite and continue to be very poorly understood. Therefore, a challenge with live parasites remains the most relevant and meaningful approach to testing the efficacy of experimental malaria vaccines both in animal models and in clinical trials. In the mouse models of *P. berghei* and *P. yoelii*, parasites are most commonly delivered by intravenous injection. This route, however, is highly artificial and produces inconsistent challenge results due to variations in the quality, purity and virulence of the inoculum produced by different laboratories on different days. In this study, we first optimized the intravenous (IV) delivery challenge model and compared it to an optimized single-mosquito bite challenge model. The latter proved to be more reliable producing more consistent challenge results while using the natural route of parasite delivery, thus avoiding the potential mis-interpretation of vaccine efficacy as this route allows vaccine-induced antibodies to exert their effects on the parasites. A single infectious bite consistently infected mice having different genetic backgrounds without the risk of overwhelming vaccine-induced protective immune responses. Recognizing the main disadvantage of the bite challenge model, the higher labor intensity, we explored an alternative approach that still delivers sporozoites to the correct anatomical site, the subcutaneous injection model. Based on this comparative study we conclude that the frequently used IV challenge model, is highly variable and the variations in the virulence of the inoculum, if not properly monitored by the rigorous inclusion of sporozoite titration curves in each challenge trial, can lead to unacceptable variations in reported vaccine efficacies. Any conclusive evaluation of a pre-erythrocytic malaria vaccine candidate should require challenge through the natural anatomic target site of the parasite, the skin.

586

INACTIVATED *ESCHERICHIA COLI* EXPRESS PROPERLY DISULFIDE-BRIDGED *PLASMODIUM FALCIPARUM FVO* MSP1-42 FROM DIFFERENT CELLULAR LOCALIZATIONS

Heather Hosie[1], Elke S. Bergmann-Leitner[1], Jessica Trichilo[2], Clarissa Dake[2], Tim Alefantis[2], Paul Grewal[2], Vito G. DelVecchio[2], Evelina Angov[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Vital Probes, Inc., Mayfield, PA, United States

Plasmodium falciparum Merozoite Surface Protein-1 (MSP-1) is a lead vaccine candidate targeting the erythrocytic stage of malaria. MSP-1 is the major protein found on the surface of merozoites and is implicated in erythrocyte invasion by the parasite. The full length protein is 195-kDa which undergoes two proteolytic processing events, leading to four polypeptides (p82, p20, p45 and p42) that noncovalently associate with the merozoite surface. In this study, the C-terminal-most fragment, p42 from the p195, was used in an expression and delivery vaccine approach using inactivated *Escherichia coli* designated GeMI-Vax. The inactivated bacteria provided inherent pathogen associated molecular patterns (PAMPS) bypassing the requirement for the addition of adjuvant. The GeMI-Vax also served to express the target antigen to various bacterial intracellular localizations: i.e. the outer membrane and the periplasmic space. Studies investigating the types of immune responses induced by presenting antigen at different sites have taught us that the location of the antigen will direct the immune responses towards either primarily cellular or humoral responses. Antigen targeting to either the outer membrane or the periplasm required construction of fusion proteins with either peptidoglycan associated lipoprotein (PAL) or the maltose binding protein (MBP), respectively. Data will be presented demonstrating proper folding of the MSP1-42-fusion proteins using disulfide-dependent monoclonal antibodies on Western blots and that the proteins are targeted to the specified bacterial intracellular localization using immunofluorescence staining. In addition, antibody responses from rabbits immunized with periplasm and outer membrane MSP1-42 GeMI-Vax will be assessed. Results from measurements of antibody fine specificities and functional activities against the parasite will be evaluated by ELISA, a bead-based multiplex Luminex assay and by the pLDH GIA, respectively. This data will demonstrate that particle-based presentation; proper MSP1-42 folding and adjuvant characteristics are important for the induction of protective antibody responses to MSP1, and are provided by GeMI-Vax.

587

VAR2CSA DBL4 AND DBL5 DOMAINS AS VACCINE CANDIDATES FOR PLACENTAL MALARIA

Tracy Saveria[1], Andrew Oleinikov[1], Gladys Keitany[1], Richa Chaturvedi[1], Joe Lograsso[1], Michal Fried[1], Patrick Duffy[2]

[1]Seattle Biomedical Research Institute, Seattle, WA, United States, [2]National Institutes of Health, Bethesda, MD, United States

Placental malaria (PM) is characterized by infected erythrocytes (iRBC) that selectively bind to chondroitin sulfate A (CSA) and sequester in placental tissue. Var2CSA, a PfEMP1 protein family member, is expressed on the surface of placental iRBCs and mediates adherence to CSA on the surface of syncytiotrophoblast. Var2CSA is a 350kD transmembrane protein that contains 6 Duffy Binding Like (DBL) domains which might contribute to the specific adhesive properties of iRBC. Here we use 3d7 Var2CSA DBL domains expressed in *E.coli* to generate antibodies specific for this protein. We show that DBL4 and DBL5 protein bind selectively to CSA *in vitro*, but not to CSC or Hyaluronic Acid, and that this binding can be inhibited by competition with sera from multigravid women. Flow cytometry results show that antisera generated against DBL4, DBL5 and a double domain of DBL4&5 bind to maternal isolates and lab strains selected for CSA binding, but not to children's parasites. These antibodies also inhibit parasite binding to purified CSA, and at least partially inhibit binding to placental tissue. The ability to generate functional antisera to pregnancy parasites via a large-scale and efficient system such as *E.coli* is an essential asset to the design of a vaccine against PM.

588

OPENING THE DOOR: CRYOPRESERVED PRIMARY HUMAN HEPATOCYTES AND THEIR POTENTIAL USE IN *IN VITRO PLASMODIUM FALCIPARUM* LIVER STAGE INFECTION MODELS

Michael Zyzak[1], Xiaoyan Zou[1], Brent House[2], Thomas Richie[1]

[1]United States Military Malaria Vaccine Program, Navy Medical Research Center, Silver Spring, MD, United States, [2]Navy Medical Research Unit #3, Cairo, Egypt

Functional liver stage assays, such as the inhibition of sporozoite invasion (ISI) assay and the inhibition of liver stage development assay (ILSDA) assess the impact of immunological responses on malaria parasite development *in vitro*, and thus potentially could identify immunological correlates of protection. However, the traditional ISI and ILSDA assays using HepG2 and HC04 cell lines are limited by low sporozoite invasion rates and by the difficulty of accurately counting invaded parasites using microscopy. In early 2009, we identified a commercial source of cryopreserved primary human hepatocytes (CPHH) and have validated their usefulness in both assays using a quantitative real time PCR approach. Advantages: (a) CPHH provides a 7-13 fold improvement in invasion rates;

(b) CPHH should be more biologically relevant for measuring functional antibody or any other aspect of liver stage biology being studied, due to the loss of normal hepatocyte biological characteristics associated with hepatoma cell lines; (c) CPHH derived from a single human liver can be purchased in sufficient quantities to conduct thousands of assays, thereby providing a standardized reagent; (d) CPHH are available across a wide demographic: male/female, multiple ethnicities, and diverse age groups including infants. Thus, CPHH significantly improve liver stage infection models allowing quantification and standardization of functional assays when coupled with a PCR-based read-out.

589

PLASMODIUM FALCIPARUM CIRCUMSPOROZOITE PROTEIN SYNTHETIC REPEAT PEPTIDE CONJUGATED TO OUTER MEMBRANE PROTEIN COMPLEX GENERATES A FUNCTIONAL IMMUNE RESPONSE IN MICE AND RHESUS MONKEYS

Craig Przysiecki[1], Bob Lucas[1], Robert Mitchell[2], Daniel Carapau[2], Zhiyun Wen[1], Hui Xu[1], Xin-Min Wang[1], Debbie Nahas[1], Robert Hepler[1], Elizabeth Ottinger[1], Jan ter Meulen[1], David Kaslow[3], John Shiver[1], Elizabeth Nardin[2]

[1]West Point Vaccines Research, Merck Sharp and Dohme Corp., West Point, PA, United States, [2]New York University School of Medicine, New York, NY, United States, [3]Research Medicine, Merck Sharp and Dohme Corp., Upper Gwynedd, PA, United States

Vaccines based on the repeat region of P*lasmodium falciparum* circumsporozoite protein (PfCSP) can elicit protective immunity in human and murine experimental hosts. The first pediatric malaria vaccine to enter Phase III trials, RTS,S which is comprised of a *Pf*CSP repeats and C-terminal fragment, elicits protection against clinical disease in immunized African children that was correlated with anti-repeat antibodies and possibly CD4+ T cell responses. The first repeat peptide-conjugate vaccine, (NANP)3-TT, elicited anti-repeat antibodies that could protect a few immunized volunteers; however titers were suboptimal. Outer membrane protein complex (OMPC) derived from *N. meningitidis* has been used successfully as a carrier for polysaccharide vaccines in infants and conjugation to OMPC increased immunogenicity of a malaria Pfs25 transmission blocking vaccine in mice and monkeys. We evaluated immunogenicity of an alum-adsorbed (NANP)6-OMPC vaccine in mice and Rhesus monkeys. BALB/c and C57Bl mice immunized with alum-adsorbed (NANP)6-OMPC developed high anti-repeat peptide ELISA titers and IFA titers using *P. falciparum* sporozoites, as well as anti-CSP reactivity with viable sporozoites. Murine immune sera inhibited invasion of human hepatoma cells by transgenic *P. berghei* sporozoites that express *P. falciparum* repeats. Vaccinated mice challenged by mosquitoes infected with transgenic parasites, demonstrated sterile immunity or delayed prepatent period and reduced parasite burden in the liver (>90% inhibition by real-time PCR). Monkeys immunized with two doses of (NANP)6-OMPC formulated with alum and a co-adjuvant developed anti-repeat antibodies that persisted at decreasing levels for 662 days. A third injection of (NANP)6-OMPC at day 662 boosted ELISA titers to peak levels observed post second dose. Rhesus sera obtained post second and third dose displayed sporozoite neutralizing activity in the parasite hematoma cell invasion assay. Results obtained in immunized mice of different MHC haplotype and a non-human primate species suggests that peptide-OMPC conjugates, based on a human acceptable carrier, may lead to new vaccine candidates.

590

EFFICACY OF FMP2.1/AS02A AGAINST GAMETOCYTEMIA IN 1-6 YEAR OLD CHILDREN IN BANDIAGARA, MALI: IMPLICATIONS FOR MALARIA ELIMINATION

Matthew B. Laurens[1], Mahamadou A. Thera[2], Drissa Coulibaly[2], Abdoulaye K. Kone[2], Ando B. Guindo[2], Dapa A. Diallo[2], Karim Traore[2], Amadou Niangaly[2], Amagana Dolo[2], Youssouf Tolo[2], Mahamadou S. Sissoko[2], Bourema Kouriba[2], Kirsten E. Lyke[3], Shannon Takala-Harrison[3], William C. Blackwelder[3], Yukun Wu[3], Johan Vekemans[4], Joe Cohen[4], David E. Lanar[5], Sheetij Dutta[5], Carter L. Diggs[6], Lorraine Soisson[6], D. Gray Heppner[5], Ogobara K. Doumbo[2], Christopher V. Plowe[1], Idrissa Traore, Issa Diarra, Modibo Daou, Mady Sissoko, Olivier Godeaux, Marie-Claude Dubois, Ripley Ballou, Darby Thompson, Tina Dube, Brent House, Jason W. Bennett

[1]Howard Hughes Medical Institute/Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States, [2]Malaria Research and Training Center, University of Bamako, Bamako, Mali, [3]Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States, [4]GlaxoSmithKline Biologicals, Rixensart, Belgium, [5]Division of Malaria Vaccine Development, Walter Reed Army Institute of Research, Silver Spring, MD, United States, [6]Malaria Vaccine Development Program, United States Agency for International Development, Washington, DC, United States

Malaria vaccines could affect malaria transmission in a community by influencing gametocyte density in human hosts in either direction, but efficacy against gametocyte carriage is not typically measured in vaccine trials. The malaria vaccine candidate FMP2.1/AS02A was recently evaluated in Bandiagara, Mali, West Africa, showing limited overall efficacy against first and multiple clinical episodes but high allele-specific efficacy. To determine the potential impact of a blood stage vaccine on malaria transmission, we evaluated the efficacy of the FMP2.1/AS02A vaccine against gametocytemia. Four hundred healthy children aged 1-6 were randomized 1:1 to receive three doses of 50 µg of FMP2.1 in 0.5mL of AS02A or rabies vaccine, 30 days apart. Malaria smears were read for all participants at scheduled time points and at unscheduled clinic visits when children presented with any malaria symptom. *P. falciparum* gametocytemia rates will be compared among both vaccine groups at scheduled time points. As a measure of cumulative gametocyte density, data from all malaria smears will be used to calculate the median area under the curve of gametocyte as well as asexual parasite density. The median area under the curve of asexual parasite density was 168,577 per microliter in the malaria vaccine group and 376,863 per microliter in the control group in the intention-to-treat analysis, and 97,708 per microliter in the malaria vaccine group and 308,638 in the control group in the according-to-protocol data set (P=0.012 in both cases). Results for gametocytemia are being completed and will be presented. In conclusion, in the context of malaria elimination, malaria vaccine candidates should not only be evaluated for efficacy against clinical episodes, but also against malaria transmission in a community. Gametocytemia rates can serve as a surrogate for malaria transmission in a community and should be part of evaluation of malaria vaccine efficacy.

591

DRASTIC REDUCTION OF MALARIA TRANSMISSION AND SEVERE CASES IN DEPARTEMENT OF OUÉMÉ AFTER PROTECTION OF COMMUNITIES AGAINST PYRETHROID RESISTANT ANOPHELINES WITH FICAMR M (BENDIOCARB 800G/KG) IN INDOOR RESIDUAL SPRAYING (IRS) IN REPUBLIC OF BENIN

Akogbeto Codjo Martin, Padonou Gil, Yadouleton Anges, Kiti Eugène

Entomological Research Center of Cotonou, Cotonou, Benin

Despite numerous efforts to roll back malaria, this disease unfortunately remains the primary cause of morbidity and mortality in children under 5 years in Africa. Despite this result, the international community decided to increase technical and financial support for malaria elimination. This is why, since 2004, the American government, through "President's Malaria Initiative" (PMI), is giving an important support to National Malaria Control Programs (NMCP) for malaria control in Africa. A large scale of IRS has been implemented with PMI support since 2008 in Oueme region (Bénin), an area characterized by a high resistance of *Anopheles gambiae* to pyrethroids. The goal of this of study is to verify if the use of a non-pyrethroid for IRS can reduce malaria transmission by eliminating *An. gambiae* populations resistant to pyrethroids and induce a significant decrease of malaria incidence. Houses of more than 350,000 inhabitants were treated with FicamR M (bendiocarb 800g/kg) because of the presence of a high permethrin resistant population of *Anopheles gambiae* (kdr frequency > 0.70). Houses were treated by the Oueme local community after training by the RTI team. The monitoring evaluation of the operation was focused on acceptability of community for IRS, quality control of the spraying done by local community, residual effect of bendiocarb, dynamics of pyrethroid resistance in areas under IRS, dynamics of malaria transmission and evolution of severe malaria in health centres. Acceptability and adhesion of communitys to the strategy was total. People who refused the strategy during the first round of IRS were convinced by the lethal action of bendiocarb on resistant mosquitoes. The spraying carried out by the community was perfect. Two weeks after IRS, 100% mosquitoes were killed whatever the strain or the position of the cone-test on the treated walls. For malaria vectors, after IRS, the human bite rate (HBR) and the inoculation rate (EIR) have drastically decreased with 94.4% reduction. This reduction due to the lethal action of bendiocarb was observed in all districts. At the same period, the managers of health centres reported 70% reduction of severe cases of malaria.

592

MANAGING DENGUE VECTOR RESISTANCE TO TEMEPHOS WITH COMMUNITY SUPPORTED APPLICATIONS OF THE MICROBIAL INSECTICIDE, VECTOBACR WG (*BACILLUS THURINGIENSIS ISRAELENSIS* STRAIN AM 65-52)

Ngan Chantha, To Setha, **Doung Socheat**

National Malaria Center, Phnom Penh, Cambodia

Cases of dengue fever transmitted by the mosquito *Aedes aegypti* in Asia are growing, and now results in an average of 12,000 cases in Cambodia since 2006. Historically, applications of temephos to larval habitats provided acceptable dengue control; however, mosquito resistance to temephos has been observed in some localities of some provinces in the recent years in Cambodia. To better manage dengue situation, our center evaluated other vector control tools. A 4 year field study with WHOPES reviewed biolarvicide, VectoBac® WG (Bti strain AM 65-52) has shown that a single direct application at 8 g/1000 L in any water type during the low dengue vector season significantly reduced the adult mosquito density in the peak season for 3 months. In Kandal province, community acceptance of VectoBac® WG was measured by treating 461,693 containers in 64,241 household in 2007. A post treatment survey showed high household acceptance (96 %) because of the quick kill in the larvae and the treated waters did not have any physical change (odor and color). Excellent product efficacy & community acceptance in Cambodia, together with successful dengue control programs with VectoBac® WG in Asia and Brazil led the Ministry of Health to include this tool in the National Dengue Control Program this year. We report here the initial results of provincial-wide, community-supported program to reduce dengue cases through vector control using VectoBac® WG initiated in May 2010.

593

THE IMPORTANCE OF INSECTICIDE RESISTANCE MANAGEMENT IN THE CONTROL OF THE MOSQUITO VECTORS OF MALARIA

Mark A. Hoppé

Insecticide Resistance Action Committee, Stein, Switzerland

Insecticides have been extensively and successfully used since the 1940s to control the mosquito vectors of disease, and have been a vital component in the fight against malaria. Indeed, vector control is considered the mainstay of malaria control programmes. However, insecticide resistance has developed in populations of the major mosquito vector species to the classes of insecticide currently recommend for vector control. As insecticide resistance continues to develop and spread, there is a real danger that these valuable interventions will be lost. This paper outlines the principles of Insecticide Resistance Management (IRM), in the vector control context. Special emphasis is placed on the need to use insecticide resistance monitoring methods that provide information that enables the decision-makers within a vector control programme to choose the intervention that best fit both their circumstances, and the principles of IRM. Much energy is currently being spent with the aim of developing, or repurposing, insecticides with novel modes of action for vector control. A strategy must be put in place to preserve the long term utility of novel insecticides, as they are developed, and take steps to maintain the effectiveness of those insecticidal tools currently available. Recommendations for such strategies are outlined. With malaria elimination returning to the international agenda, it is argued that only through IRM can the sustainable use of insecticidal vector control interventions be maintained.

594

DEVELOPMENT OF A GRAVID-OVITRAP FOR COLLECTING *AEDES AEGYPTI*

Roberto Barrera, **Andrew J. Mackay**, Manuel A. Amador, Belkis Z. Caban, Veronica Acevedo, Gilberto Felix

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

The ovitrap has been used for many decades as a very sensitive and inexpensive tool for detecting the presence of *Aedes aegypti*. Ovitraps also have been used to reduce the reproductive potential of female mosquitoes entering the trap by providing a suitable substrate for oviposition, while preventing eggs deposited from producing adult mosquitoes. Sticky ovitraps and lethal ovitraps (ie. gravid-ovitraps) incorporate an additional mechanism to kill or capture gravid mosquitoes. To be a practical surveillance or control tool, a gravid-ovitrap must be effective, inexpensive, and shouldn't require frequent maintenance. Traps that don't utilize pesticides are more likely to be accepted by homeowners concerned with potential health/environmental risks associated these chemicals, and won't contribute to the development of insecticide resistance. Our objective was to develop a trap that incorporates simple, low-cost mechanisms for eliminating gravid *Ae. aegypti* and their progeny, that doesn't use a toxic insecticide, and that can remain efficacious for an extended period of time without servicing. We compared seven commercial adhesives for capturing adult *Ae. aegypti* and determined that the most promising adhesive can be used in a gravid-ovitrap for at least six weeks under field conditions without a significant loss of capture efficacy. We established that a gravid trap baited with hay infusion and a supplemental hay packet remains very attractive to gravid *Ae. aegypti* for at least three weeks. Data collected from field tests of gravid-ovitraps in metropolitan San Juan suggest an

optimal trap density of three traps per home, with a mean capture rate of 1.4 *Ae. aegypti* females per trap per day. We also evaluated a synthetic polymer that is highly attractive to gravid *Ae. aegypti* as an oviposition substrate, but prevents development of their progeny. Our results indicate that a gravid-ovitrap incorporating these components (adhesive, supplemental hay and artificial oviposition substrate) could be an effective tool for the surveillance and/or control of *Ae. aegypti*.

595

FIELD TRIALS OF A NEW GRAVID-OVITRAP FOR INTEGRATED AREA-WIDE CONTROL OF *AEDES AEGYPTI* IN PUERTO RICO

Roberto Barrera, Andrew J. Mackay, Manuel A. Amador, Belkis Z. Caban, Veronica Acevedo, Gilberto Felix

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

A new gravid-ovicidal trap was used in conjunction with source reduction and larviciding for integrated area-wide control of *Aedes aegypti* in an isolated urban community (295 houses) in southern Puerto Rico (trap intervention area; TIA). Mosquito population post-intervention was compared with pre-intervention value in TIA and with change in a nearby isolated urban community (423 houses) where only source reduction and larviciding were concurrently applied (no trap intervention area; NTIA). The trap used hay infusion and color contrast as attractants for gravid females that were then captured on internal adhesive surfaces. The trap also used a synthetic polymer as a substrate for egg laying instead of water, where hatching larvae fail to develop. Three traps were deployed for two months in each home in TIA. Source reduction consisted of a clean-up campaign and turning over containers that could not be disposed of or treated with a larvicide (three formulations of spinosad). Containers holding animal or human drinking water were left untreated. Water storage containers were not common in the study areas. The number of adult female *Ae. aegypti* in each community was monitored using 28 BG-Sentinel TM mosquito traps in TIA and 40 in NTI. These traps used BG-lure and were operated for three days a week during eight weeks, before and after the intervention. Average *Ae. aegypti* female post-intervention reduction was 43% in TIA and 21.7% in NTIA. Mosquito population reduction due to the gravid-ovicidal traps was 21%. Three gravid-ovicidal traps captured an average of 0.54 *Ae. aegypti* females per house per day (>95% gravid or parous). The number of eggs per captured female in the traps was 14.9. Increasing trap attractiveness is a next step in the development of this low-maintenance, inexpensive device that targets the epidemiologically important, gravid/parous *Ae. aegypti* females.

596

THE CARTAGENA PROTOCOL AND GENETICALLY MODIFIED MOSQUITOES

John M. Marshall

California Institute of Technology, Pasadena, CA, United States

The Cartagena Protocol on Biosafety is the fundamental document of the United Nations on the responsible use of genetically modified (GM) organisms. The Protocol applies to GM mosquitoes; however its terms were negotiated primarily with concerns over the safety and trade of GM crops in mind. We argue that, while the Protocol may be adequate for strains of GM mosquitoes intended for population suppression, it is inadequate for strategies intended to replace entire mosquito populations with disease-resistant varieties. In this latter strategy, gene drive systems are being considered that are capable of propagating transgenes within and across national borders. In its current form, the Protocol provides inadequate protection against an accidental release, notably due to the exemption of GM mosquitoes in transit or destined for contained use from the Advance Informed Agreement. At the same time, the conditions for an intentional release are almost impossible to satisfy, requiring unanimous approval from every country that the species inhabits. Furthermore, mosquitoes infected with non-transgenic *Wolbachia* bacteria are exempt from the Protocol, despite unknown consequences for the environment and human health. We encourage future regulation that addresses the unique biosafety concerns of modified mosquitoes and seeks a balance between the precautionary principle, respect for the sovereignty of states, and the ethical mandate to prevent disease on a global scale.

597

SUITABLE MATING COMPETITIVENESS OF INCOMPATIBLE *AEDES POLYNESIENSIS* MALES SUPPORTS LYMPHATIC FILARIASIS ELIMINATION STRATEGY FOR THE SOUTH PACIFIC

Limb K. Hapairai[1], Eric Chambers[2], Bethany Peel[2], Stephen Dobson[2], Hervé Bossin[1]

[1]Institut Louis Malardé, Papeete, Tahiti, French Polynesia, [2]Department of Entomology, University of Kentucky, Lexington, KY, United States

Despite the sustained mass drug administration (MDA) of anti-filarial treatments over several decades, lymphatic filariasis (LF) remains a leading cause of disability in the South Pacific. Recent epidemiological observations clearly demonstrate that MDA alone will not be enough to end the LF transmission cycle, at least in some Pacific island countries. Supplemental control strategies are thus much needed to ensure the success of the global LF elimination campaign. Obligate vector mosquitoes provide additional targets that can complement existing anti-filariasis approaches. However, due to the ubiquitous nature of mosquitoes, conventional methods have failed to successfully control the vector Aedes polynesiensis, the primary LF vector throughout most of the South Pacific. Such paucity in the arsenal of tools available to control Ae. polynesiensis has raised interest in the use of evolutionary genetics to fight vector-borne diseases. Replacing the endosymbiont *Wolbachia* present in Ae. polynesiensis with that from Ae. riversi through interspecific hybridization and introgression has led to the development of a laboratory strain (CP) which is bi-directionally incompatible with its wild counterpart, resulting in egg sterility. Laboratory assays demonstrated the equal competitiveness of CP males and established the proof-of-principle of population elimination following the introduction of incompatible males into wild type A. polynesiensis cage colonies. CP male competitiveness was assessed in a field cage trial. This bioassay demonstrated equal CP male mating competitiveness with their wild counterpart under semi-natural tropical conditions. These findings support the implementation of a large field trial to assess the efficacy of the *Wolbachia* -mediated mosquito suppression strategy as a supplemental strategy to curb LF prevalence in endemic regions of the South Pacific.

598

INSECT REPELLENTS: FROM MODE OF ACTION TO NEW APPLICATIONS IN VECTOR CONTROL

Cédric Pennetier[1], Bruno Lapied[2], Fabrice Chandre[1], Vincent Corbel[3]

[1]Institut de Recherche pour le Développement, Montpellier, France, [2]RCIM, Université d'Angers, Angers, France, [3]Institut de Recherche pour le Développement, Cotonou, Benin

With the spread of pyrethroid resistance in most mosquito vector species and the lack of alternative compounds for public health issues, the search for alternative control strategies effective against resistant vector populations has become a priority. In this weapons race, insect repellents, which have been used for some time via topical application to the skin, are becoming of greater interest for community protection against mosquito borne diseases. Here we used a multidisciplinary approach to investigate whether repellents could be used to limit the contact between mosquito vectors and humans. First, using electrophysiological, biochemical and toxicological methods, we described two modes of action of the gold standard repellent DEET: it is an acetylcholine esterase inhibitor and it exerts neurotoxic effects through an elevation of the intracellular calcium concentration. We also showed that repellants had strong synergistic effects with available insecticides and have great potential for use in insecticide for insecticide treated nets or indoor residual sprayings.

Moreover we showed in both the laboratory and field that different fabrics impregnated with repellents alone and combined with other biocides are highly effective against resistant mosquito vectors. We also showed that the impregnation of clothes with repellents is also a valuable opportunity for personal protection. Moreover repellents could be used for their primary activity, as an insect behavior modifier, in promising strategies like the push-pull strategy. To conclude, repellents are highly promising to better control pyrethroid resistant vectors. Although the volatility of these chemicals limits their immediate use on long lasting fabrics, overcoming this technological problem should be lot more easily achieved than finding insecticides with new modes of action.

599

DRY SEASON PILOT INDOOR RESIDUAL SPRAY (IRS) TARGETING RIVERBANK HAMLETS IN SUDAN SAVANNA AREAS OF MALI

Nafomon Sogoba[1], Moussa Keita[1], M'Bouye Diallo[1], Ibrahima Baber[1], Massiriba Kone[1], Nicholas C. Manoukis[2], Ellen Dotson[3], Sekou F. Traore[1], Seydou Doumbia[1]

[1]MRTC/FMPOS University of Bamako, Bamako, Mali, [2]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States

In the Sudan savanna of Mali there is marked seasonality in malaria transmission, a consequence of very low densities of mosquito vectors during the dry season, and so control efforts are generally focused on the rainy season. However, there are parts of that environment, such as the riparian parts of the Niger River, where there is perennial breeding of *Anopheles gambiae* s.l. together with dry season malaria transmission. Thus control measures targeting adult mosquitoes during the dry season in these areas are of interest, and may decrease the size of mosquito population and transmission in the subsequent rainy season. This study aims to explore the effectiveness and potential impact of a dry season IRS in hamlets along the Niger River where mosquitoes continue breeding in the dry season and describe ways in which such an approach might limit malaria transmission during the rainy season. Entomological parameters of malaria transmission were monitored using PSC before and after the IRS in 3 sets of hamlet-inland villages in 2008 and 2009, respectively. Mosquito density and entomological inoculation rate (EIR) in 2009 (after IRS) were lower than that of 2008 (before IRS) in the 3 hamlets under study (Bozokin, Fourda and Somonosso). The geometric mean number of mosquitoes per house during the rainy season showed a reduction of 40.0% in Bozokin [1.5 (0.6_2.4) vs 0.9 (0.3_1.5)]; 8.3% in Fourda [1.2 (0.5_1.8) vs 1.1 (0.6_1.6)], and 33.3% in Somonosso [1.8 (1.2_2.5) vs 1.2 (0.5_1.8)]. The same pattern was observed in EIR, measured as the number of infective bites per person per season, with a reduction rate of 79.4% (0.34 vs 0.07), 36.4% (1.87 vs 1.19) and 42.9% (0.28 vs 0.16) respectively in Bozokin, Fourda and Somonosso. Mosquito density and EIR decreased between 2008 and 2009 in hamlets where the IRS was performed. However excepting in Bozokin, this reduction was < 50%. Additional dry season IRS intervention may be required to observe any significant reduction in malaria transmission in subsequent rainy season.

600

IMPACT OF LONG-LASTING DELTAMETHRIN-TREATED CONTAINER COVERS ON *AEDES AEGYPTI* OVIPOSITION

Daniel Kline[1], Gary G. Clark[1], Joyce Urban[1], Frances Golden[1], Michael B. Nathan[2], Tessa Knox[3]

[1]United States Department of Agriculture/ARS, Gainesville, FL, United States, [2]World Health Organization, Geneva, Switzerland, [3]Vestergaard Frandsen, Nairobi, Kenya

USDA researchers are studying novel methods to control *Aedes aegypti*. One approach focuses on prevention of *Ae. aegypti* oviposition. In collaboration with Vestergaard Frandsen Ltd., factory-treated long lasting deltamethrin PermaNet® Container Covers (jar lids) were evaluated with 55-gallon drums with and without covers. Exclusion efficacy was measured with sticky ovitraps and oviposition substrates placed on the inner wall of the drums. Tests were performed in 1,800 ft2 outdoor screened cages (30 ft wide, 60 ft long, 16 ft high gabled to 18 ft) at the USDA CMAVE facilities in Gainesville, Florida. In test 1, there was 1 drum per cage with either an untreated or deltamethrin-treated cover, or an uncovered drum (untreated control). In test 2, there were 4 drums per cage: 3 covered and 1 uncovered drum, 1 covered and 3 uncovered drums, or 4 uncovered drums (untreated control). Test 3 was similar to test 2 but with a different version of the Container Cover. For each test, 200 gravid *Ae. aegypti* were released into each cage. The drum(s) were 2/3 full of well water and lined with absorbent germination papers to detect female oviposition. Container Cover efficacy was measured 24 hrs post-release of females with 5 widely distributed sticky ovitraps (containing a 10% 7 day-old hay infusion) placed in each cage as alternative oviposition sites for gravid females. Sticky ovitraps were examined after 48 hrs and egg (germination) papers were removed after 72 hrs. Drums with untreated covers yielded a similar number of females to those with no cover, whereas treated covers resulted in a 64% reduction in females. With 1 of 4 drums with treated covers, there was a 45-65% reduction in females and a 42-52% reduction in oviposition. With 3 of 4 drums with treated covers, there was a 67-100% reduction in females and a 75-100% reduction in oviposition. The presence of treated Container Covers of either version significantly reduced female oviposition. Container Covers present a potential tool for the control and prevention of dengue virus transmission.

601

MONITORING THE DURABILITY OF LONG-LASTING INSECTICIDAL BEDNETS IN RURAL ETHIOPIA

Stephen C. Smith[1], Aprielle Brackery[2], Gedeon Yohannes[3], Patricia M. Graves[2], Tekola Endeshaw[3], Estifanos B. Shargie[3], Aryc W. Mosher[2], Paul M. Emerson[2], Teshome Gebre[3]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]The Carter Center, Atlanta, GA, United States, [3]The Carter Center, Addis Ababa, Ethiopia

Following the distribution of 3 million PermaNet™ 2.0 long-lasting insecticidal nets in Ethiopia in February - May 2007, a total of 609 nets were collected from households at three time intervals: 2007, 2008, and 2009; to assess physical damage and insecticide loss, and to develop practical methods to quantify deterioration. Nets were collected from 19 sites and time-in-use ranged from 3 to 32 months. Collected nets were confirmed as being from the 2007 campaign by using the batch number printed on each net's label. Physical deterioration was first assessed by counting and measuring the size and location of each hole, and later by counting the number of holes falling into each of three size categories. Insecticide retention was measured by x-ray fluorescence spectrometry, and confirmed by testing a subset of nets using high-performance liquid chromatography. Hole formation began early: over 40% of nets used for 3 months had at least one hole >0.5 cm in diameter. The number of holes per net (hole rate) followed a highly skewed distribution, with many nets having few holes and a few nets having many holes. Median hole rate increased from 1 for nets used for 3-6 months to 10.5 for nets used for 17-21 months. Pairwise analysis showed that nets collected from 5 pairs of sites differed significantly ($p<0.002$) in hole rates after 17-21 months of use. The distribution of hole sizes was highly skewed and, although hole rate increased with time-in-use, the ratio of large to small holes remained unchanged from 3-6 months to 17-21 months. Repairs were rare, suggesting that net lifetime could be increased significantly by improved user care.

Insecticide analysis indicated that 96% (192/200) of the nets retained sufficient (>10mg/m2) deltamethrin after 28-32 months of use. The distribution of insecticide level was very broad with 0.5% (1/189) having inadequate deltamethrin after 3-6 months and 6% (12/200) after 17-21

months. Pairwise analysis of the insecticide levels of nets used for 17-21 months found that 3 pairs of collection sites had nets with significantly different levels of insecticide ($p<0.002$).

602

THE GENETIC BASIS OF PYRETHROID RESISTANCE IN THE MAIN AFRICAN MALARIA VECTOR *ANOPHELES GAMBIAE* S.S.

Claudia Witzig[1], Strode Clare[1], Rousseau Djouaka[2], Charles Wondji[1], Hilary Ranson[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]IITA Cotonou, Cotonou, Benin

Vector control measures for malaria rely heavily on insecticides. Increasing usage of insecticides, has led to the emergence of resistance to all of the classes of insecticides currently available for public health. This is threatening the success of vector control programmes. Quantitative trait loci (QTL) mapping is being used to identify the mechanisms responsible for pyrethroid resistance in *Anopheles gambiae* in Benin. F2 populations of *An. gambiae*, raised directly from field collected mated females, have been analysed to identify the major loci controlling resistance. New genetic markers within candidate genes have been identified to complement the existing sets of SNP and microsatellite markers. Preliminary analysis identified one QTL on chromosome 3R near a P450 cluster. Advanced Intercross Line (AILs) are now being used to finesse the mapping. Both target site and metabolic resistance have been reported in the M form of *An. gambiae* in Southern Benin and this genetic approach will enable us to determine the relative contribution of different alleles to the resistance phenotype. Identification of responsible factors will hopefully lead to a better understanding of the resistance mechanism and enable the development of more powerful insecticides as well as suitable screening assays to detect resistance in the early stages of development.

603

HEALTH IMPACT ASSESSMENT OF DEVELOPMENT PROJECT: IMPACT OF SARDAR SAROVAR NARMADA PROJECT ON MOSQUITO-BORNE DISEASES IN GUJARAT, INDIA

Harish Chandra Srivastava[1], R.M. Bhatt[2], R.S. Yadav[3]

[1]National Institute of Malaria Research, Nadiad, Gujarat, India, [2]National Institute of Malaria Research, Field Station (Near MHI Hospital), Raipur, Chhatisgarh, India, [3]Vector Ecology and Management, Department of Control of Neglected Tropical Diseases, World Health Organization, Geneva, Switzerland

Sardar Sarovar Narmada in Gujarat is a multipurpose water resources development project on river Narmada in Narmad district. Assessment of the impact of this water development project on mosquito borne diseases has been undertaken in command area of Phase I and II districts. We studied geographical reconnaissance of mosquito breeding habitats, bionomics of vectors assessment of the incidence of malaria, dengue and filarial and links between Agro-ecosystems in above said areas. In general malaria in villages in command area remained low as compared to non-command areas in 2009. The mosquito breeding habitats was surveyed along the main canal across 6 districts in phase I and II. *Anopheles culicifacies* which is the main malaria vector in the plains was found to breed in almost all the habitats surveyed. Breeding of filariasis vector *Culex quinquefasciatus* and vector of Japanese Encephalitis *Cx. vishnui* group was also detected from different habitats. All three important malaria vectors viz., *An. culicifacies, An. stephensi* and *An. fluviatilis* were collected resting indoor from the indicator villages. Dengue vectors *Aedes aegypti* and *Ae. albopictus* were collected in very low numbers in adult collections. *An. culicifacies* was predominantly a zoophilic species represented by sibling species A, B and C of which B was predominant (88%). Paddy cultivation in command areas has increased after commencement of irrigation in phase I districts. The depth of water has begun rising in theses areas. The study is in progress in 3 more districts in phase II command area. The lessons learned from this study would be useful for incorporating health safeguards in the development of future project in the Gujarat state.

604

MALARIA TRANSMISSION IN MINERAL PROSPECTION AREAS IN THE BRAZILIAN AMAZON REGION

Izis M. Sucupira, Marinette M. Povoa

Instituto Evandro Chagas, Ananindeua, Brazil

The mineral prospection projects established in Brazilian Amazon promotes the intensity of human migration and can causes environmental changes that modify the transmission profile, since these modifications increase the vector-man contact. Our aims were to identify anopheline mosquitoes species and to determine which species were implicated on malaria transmission in two study areas. The study was performed on the municipalities of Juruti (bauxite extraction - Juruti Project) and of Marabá/Parauapebas (iron extraction - Salobo project) both in Para state. The first project was implemented on 2003 while the second one is older than 20 years. The mosquitoes collection were carried out during 12 hours (from 6 p.m to 6 a.m), 2 or 3 times/year, from 2005 to 2008. All collected mosquitoes were identified by morphological characters and used for detection of malaria parasites by ELISA, and 10% of them were dissected for reaching the parity tax. In Juruti project we had collected 976 mosquitoes of 8 species and the most important for malaria transmission, was the *Anopheles albitarsis s.l.*, which had showed a parity tax of 9.6% and was the only one found infected by *Plasmodium vivax* (infection rate = 0.1%), which is the main specie circulating among the human population in Brazil. In Salobo project we collected 746 adults mosquitoes of 11 species. From those, *An. darlingi* and *An. albitarsis s.l.*, that are incriminated as main vector in Brazil, had showed parity tax of 8,6% and 6,1%, respectively. Furthermore they were the only species founded infected by malaria parasites (*An. darlingi*: *Plasmodium falciparum* and *P. vivax* and *An. albitarsis*: *P. vivax*), resulting on a infection rate of 0.8%. Based on the results it is possible to deduce that the longer is the project more stable is the malaria transmission

605

HARMONIC CONVERGENCE AND THE SEXY SONS HYPOTHESIS IN *AEDES AEGYPTI*

Lauren Cator, Ron R. Hoy, Laura C. Harrington

Cornell University, Ithaca, NY, United States

In order to improve mosquito control programs, it is important to understand mating biology. In particular, little is known about whether mate assessment occurs and what characteristics are important for fitness. Several medically important mosquito species mate on the wing in aerial swarms. Flight tone is important for male orientation to females. Recently, we determined that variation in flight tone is perceived by both males and females. Males and females altered their flight tone to converge on common harmonic frequencies and this harmonic convergence was important for the formation of a successful copula. The studies presented here, build upon our earlier work on harmonic convergence. We recorded acoustic interactions of male and female *Aedes aegypti* prior to mating. We then followed females throughout their lifetime and measured both direct benefits to females (longevity and daily egg laying counts) and indirect benefits manifested in their offspring. Our results are consistent with the predictions of the sexy son hypothesis: male offspring of pairs that converged prior to mating had higher mating success. In addition to direct and indirect benefits of convergence behavior, we will present data on the heritability of convergence. By understanding the signals used in mating interactions, we will be better able to understand the mating system of *Ae. aegypti* as a whole.

606

FIELD EVALUATION OF THE BEHAVIORAL DIFFERENCES ASSOCIATED WITH TWO GEOGRAPHICALLY ISOLATED POPULATIONS OF *ANOPHELES DARLINGI*

Paige Sachs, Nicole Achee, Diana Riner, John Grieco

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

Anopheles darlingi is a major vector for malaria in Central and South America. A review of the literature shows that a number of independent studies have documented behavioral differences in this species across its range including differences in biting activity, feeding preference and propensity for endophagy. Previous work has also shown that *A. darlingi* can be categorized into two genotypes. Genotype 1 is found in Amazonia and southern Brazil and genotype 2 is found in Belize, Guatemala, Colombia and Venezuela. It is not known whether these different genotypes affect mosquito behavior which could contribute to differences in vector competence. The current work represents the first study to incorporate a multi country evaluation of the genetic diversity of this species with a thorough evaluation of behavioral variations in the two populations. Using an experimental hut design, the entrance and exit behavior of *An. darlingi* from two locations; Iquitos, Peru, representing genotype 1 and Cayo district, Belize, representing genotype 2, were evaluated. Differences in the endophagic behavior of this species across its range can translate into differences in its ability to transmit malaria. These behavioral differences can also impact the timing and potential impact of interventions such as long lasting bed nets and other personal protective measures (i.e. mosquito coils and insecticide treated materials).

607

MODELING *CULEX TRITAENIORHYNCHUS* MOSQUITOES TO PREDICT THE GEOGRAPHIC DISTRIBUTION OF JAPANESE ENCEPHALITIS IN THE REPUBLIC OF KOREA

Penny M. Masuoka[1], Terry A. Klein[2], Heung-Chul Kim[2], David M. Claborn[3], Nicole Achee[1], Richard Andre[1], Judith Chamberlin[1], Jennifer Small[4], Assaf Anyamba[4], Dong-Kyu Lee[5], Suk H. Yi[2], Michael Sardelis[6], John Grieco[1]

[1]Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [2]65th Medical Brigade/United States Army MEDDAC-Korea, Seoul, Republic of Korea, [3]Center for Homeland Security, Missouri State University, Springfield, MO, United States, [4]Hydrological and Biospheric Sciences Laboratory, NASA's Goddard Space Flight Center, Greenbelt, MD, United States, [5]Department of Health and Environment, Kosin University, Busan, Republic of Korea, [6]National Center for Medical Intelligence, Fort Detrick, MD, United States

Japanese encephalitis (JE) is a serious mosquito-borne disease that results in approximately 35,000 cases reported worldwide each year. The disease occurs throughout Southeast Asia, India, parts of China, Japan, and Korea. The primary JE vector, *Culex tritaeniorhynchus*, is associated with rice fields and other low-lying grassy areas that flood. Large water birds are the primary reservoir, while pigs are amplifying hosts of the virus. Partners of the Armed Forces Health Surveillance Center (AFHSC), Division of Global Emerging Infections Surveillance (GEIS) and Response System Operations are working to develop predictive models for JE surveillance for the purpose of developing mitigation strategies in Asia. As a first step in this project, an ecological niche modeling program, Maxent, was used to determine the potential distribution of *Cx. tritaeniorhynchus* in the Republic of Korea (ROK). Input data for the model included mosquito occurrence locations from field collections throughout the ROK and environmental data, including land cover, climate variables, normalized difference vegetation index (NDVI), and elevation. A probability map produced by the model predicted low probabilities of *Cx. tritaeniorhynchus* in the forested, mountainous areas and high probabilities associated with rice paddies and low-lying areas. A jackknife test demonstrated that land cover, elevation, summer NDVI, summer minimum temperature, and maximum winter and fall temperature contributed to the model for the presence of *Cx. tritaeniorhynchus*. The model was validated using traditional statistical methods, and reported JE cases from 2001 to 2009 fell within the higher probability areas on the map. Ecological niche modeling of *Cx. tritaeniorhynchus* was shown to be a useful tool for identifying areas of greater risk of transmission of JE virus. Although reservoir and amplifying hosts are necessary for transmission of JE virus, the prediction of disease outbreak occurrences may be more dependent upon mosquito abundance rather than presence. Future work will examine the use of real-time satellite data to determine if mosquito abundance can be predicted using NDVI or climate variables.

608

ASYMMETRICAL INTERSPECIFIC COMPETITION BETWEEN *CULEX RESTUANS* AND *CX. PIPIENS* IN ILLINOIS

Joel A. Morris, Robert J. Novak

University of Alabama-Birmingham, Birmingham, AL, United States

Competitive interactions between sympatric West Nile virus vectors, *Culex restuans* and *Cx. pipiens*, were evaluated in Champaign-Urbana, IL in 2007 using a replacement series in which field collected mosquito larvae were placed in rearing pans with different ratios of each species (100%:0%, 75%:25%, 50%:50%, 25%:75%, and 0%:100%). Each larval combination was repeated at biologically relevant temperatures (22°C, 26°C, and 30°C) and densities (0.05, 0.10, 0.20, and 0.40 larvae/ml). Results suggest that overall survival of *Cx. restuans* was greater than *Cx. pipiens* across all treatment variables. However, each species responded to survival and fitness (body mass) tradeoffs quite differently. Survival of both species was primarily limited by increasing density, but survival of *Cx. restuans* benefited from increasing competition with *Cx. pipiens* while survival of *Cx. pipiens* benefited from intermediate temperatures. In contrast, while fitness of both species primarily decreased with increasing densities, only *Cx. pipiens* mass benefited from increasing competition with *Cx. restuans*. Thus, *Cx. restuans* placed emphasis on survival at the expense of fitness, while *Cx. pipiens* placed emphasis on fitness at the expense of survival rate. These species yielded identical total biomasses by sex, which suggests that superior competition for *Cx. restuans* may be countered by superior fitness for *Cx. pipiens*. This would allow robust populations *Cx. pipiens* to rapidly colonize opened niches as *Cx. restuans* enter diapauses in July.

609

CLIMATE AND LAND COVER INFLUENCES ON *CULEX TARSALIS* (DIPTERA: CULICIDAE) POPULATIONS IN SIOUX FALLS, SOUTH DAKOTA

Ting-Wu Chuang[1], Michael B. Hildreth[2], Denise L. VanRoekel[3], Lon Kightlinger[4], Michael C. Wimberly[1]

[1]GISc Center of Excellence, South Dakota State University, Brookings, SD, United States, [2]Department of Biology and Microbiology, South Dakota State University, Brookings, SD, United States, [3]Sioux Falls Health Department, Sioux Falls, SD, United States, [4]South Dakota Department of Health, Pierre, SD, United States

West Nile virus (WNV) first invaded the Northern Great Plains (NGP) in 2002, and the incidence of human cases in this region has remained high compared to the rest of the United States. *Culex tarsalis* is the most important vector of WNV infection in the NGP, and the abundance of mosquitoes is a key factor in the amplification and transmission of WNV. However, the effects of land cover and climatic variability on vector populations in the NGP are not well understood. This study compared *Cx. tarsalis* populations and examined their relationships with land cover types, temperature, and precipitation in Sioux Falls, South Dakota from 2005 to 2008. Between 20 and 40 CDC CO2-baited light traps were set annually in Sioux Falls from May to September of 2005 through 2008,

and the number of *Cx. tarsalis* was identified by seasonal staff. Land cover characteristics were acquired from the 2001 National Land Cover Dataset (NLCD) and the percentages of selected land cover types were calculated within a buffer zone around each trap determined by the flying range of *Cx. tarsalis*. Temperature and precipitation were summarized from local weather stations. Land cover analysis indicated that wetland and cultivated crops were usually positively correlated with mosquito populations but the strength and seasonality of these correlations varied by year. Developed land showed consistent negative associations through the whole study period. Both temperature and rainfall showed lagged effects on mosquito populations. In general, higher temperature and precipitation in different week lags were associated with higher mosquito populations in the current week after adjusting for spatial autocorrelation. The early emergence of vector abundance in 2007 was associated with a high number of WNV human cases in early summer. This study demonstrated the associations among *Cx. tarsalis* populations, land cover types, and seasonal climate patterns in Sioux Falls. These results can be used to improve vector control strategies and disease prevention efforts.

610

BLOOD MEAL ANALYSIS OF MALARIA MOSQUITO VECTORS, EQUATORIAL GUINEA

Vasiliki Pappa[1], Hans Overgaard[2], Michael Reddy[1], Jeff R. Powell[1], Adalgisa Caccone[1]

[1]Yale University, New Haven, CT, United States, [2]Medical Care Development Inc./Marathon Oil Corp., Bioko, Equatorial Guinea

Host feeding patterns and longevity serve as important parameters in determining the capacity of a mosquito population to act as a vector of disease. Mosquitoes such as *Anopheles gambiae* are considered highly anthropophilic and endophilic. However, in endemic regions, vector populations consist of more than one species that may vary in terms of host seeking behavior. More importantly, in regions where vector control programs have been in effect for years, vector populations have been observed to prefer non-human hosts. The degree of anthropophily can be assessed by estimating the Human Blood Index (HBI). This index is then used to estimate the vectorial capacity in an area. In this study we used a multiplexed PCR assay to identify the bloodmeal source of the vector population in Equatorial Guinea, in order to estimate the HBI. All the vectors showed high anthropophily, with HBI greater than 0.5. Bloodmeal analyses are essential to determine vector host choice and feeding behavior changes, which highlights the importance of such studies for evaluating malaria control interventions. As a result, these analyses should be incorporated in malaria control programs.

611

DIFFERENTIAL IMPACT OF LONG-LASTING INSECTICIDE TREATED NETS ON ANOPHELINE VECTOR POPULATIONS AND MALARIA TRANSMISSION IN PAPUA NEW GUINEA

Lisa Reimer[1], Edward Thomsen[1], Gussy Koimbu[1], Absalom Mai[1], August Kapa[1], John Keven[1], Henry Dagoro[1], Ivo Mueller[2], Peter Siba[2], James Kazura[3], Manuel Hetzel[2], Peter Zimmerman[3]

[1]Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea, [2]Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea, [3]Case Western Reserve University, Cleveland, OH, United States

In Papua New Guinea (PNG) members of the Punctulatus Group, including *Anopheles punctulatus*, *An. koliensis*, *An. farauti s.s.*, *An. farauti 4* and *An. hinesorum* (formerly *An. farauti* 2), exhibit heterogeneities in distribution, biting behavior and malaria infection levels. The PNG National Department of Health recently launched a nation-wide long-lasting insecticide treated net (LLIN) program. This study aimed to evaluate the impact of the campaign on anopheline species density, composition, feeding behavior and malaria infectivity. Eight sentinel sites were chosen from 6 provinces of PNG representing coastal, riparian, inland and highland regions. Entomological surveys were conducted prior to and one year post-LLIN distribution. Two of these sites were chosen for an intensive monthly entomological evaluation beginning one year prior to distribution in August 2009 until August 2010. Host-seeking anophelines were collected by the landing catch method from 6pm to 6am (N=46,000). Adults were identified to morphospecies and confirmed by PCR-RFLP of the internal transcribed spacer 2 rDNA. Malaria infectivity was determined by circumsporozoite ELISA for *P. falciparum*, *P. vivax* 210 and *P. vivax* 247. Overall man-biting rates for each species were reduced following the LLIN campaign. The reduction ranged between five-fold and ten-fold, and was greater in species with late night biting habits such as *An. punctulatus* and *An. koliensis* than the Farauti complex which has a tendency to bite in the early evening. Within 6 months of LLIN distribution, peak biting times for both *An. farauti 4* and *An. punctulatus* shifted significantly ($p<0.001$). The proportion of *An. farauti 4* biting between 6pm and 10pm was significantly higher after bednets, while the peak biting times for *An. punctulatus* shifted from 12am-3am pre-LLIN to 11pm-1am post-LLIN. Preliminary data show that members of the Farauti complex have lower rates of infectivity to *Plasmodium* than *An. punctulatus* and *An. koliensis*. Differences in biting times and susceptibility to infection will impact the success of LLIN campaigns, and the behavioral shift to earlier biting may alter malaria transmission dynamics.

612

LATE BITING OF *AEDES ALBOPICTUS* IN CHIANG MAI PROVINCE, NORTHERN THAILAND, CHANCE FOR PREVENTION AND MOSQUITO CONTROL

Wannapa Suwonkerd[1], Nantawan Suwannachote[1], Theeraphap Chareonviriyaphap[2]

[1]Office of Disease Prevention and Control, Ministry of Public Health, Chiang Mai, Thailand, [2]Department of Entomoloy, Kasetsart Univesrity, Bangkok, Thailand

Aedes albopictus is widely distributed throughout Thailand and play an importance role in transmitting of viral diseases i.e dengue fever and chikungunya in Thailand. It breeds in natural container as well as human made container, however little is known about its behavior particularly the biting pattern. The study was conducted in rural of Chiang Mai, northern Thailand. 6 households located at head, middle and at the end of the village were selected to be the collection sites. In each household one pair of collector collecting the mosquito by using the sweeping net and aspirator, collecting mosquitoes inside and outside a house of 10 minutes interval between indoor, outdoor and brake, as a total 40 minutes collecting time in each household. The mosquito collection started from 6.00 am to 23.00 pm, two days per month. The study was conducted from January to April and May to August 2010 representing dry and wet season respectively.

In dry season (January to April) Ae. albopictus showed long day biting from 06.00 hr to 23 hr with sharp peak from 15.00-18.00. However after sunset, 18.00-23.00 hr. this mosquito showed the same number of mosquito collected between 12.00-15.00 hr. The raining season study is on going and it will be further discuss later.

613

MULTIPLE MATING IN *AEDES AEGYPTI*: SPERM TRANSFER AND USAGE PATTERNS

Prasit Deewatthanawong, Laura C. Harrington

Cornell University, Ithaca, NY, United States

Mosquito mating behavior including mating frequency is an important factor affecting mosquito population structure and genetic control efforts. Our previous work has detected a small but potentially significant frequency of multiple mating in the laboratory and field for *Aedes aegypti*. The objective of this study was to investigate the frequency of multiple mating and more specifically, the frequency of sperm transfer and

female utilization of sperm from more than one mating. In this study, we report our results of female sperm usage patterns using a combination of approaches including PCR-based detection of sperm genotypes and screening of female reproductive output.

614

BEHAVIOURAL PATTERN OF THE MALARIA VECTORS AND VECTOR CONTROL INTERVENTIONS IN LUANGWA VALLEY, SOUTHEAST ZAMBIA

Aklilu Seyoum[1], Chadwick Sikaala[2], Javan Chanda[2], Dingani Chinula[2], Neil Lobo[3], Frank Collins[3], Gerry F. Killeen[4]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]National Malaria Control Centre, Lusaka, Zambia, [3]University of Notre Dame, South Bend, IN, United States, [4]Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania

Insecticide treated nets and Indoor residual spraying are the principal vector control interventions in Africa. Understanding of the behaviour of malaria vectors in different vector control intervention areas is important for effective implementation of the control programs. Human landing catches conducted both indoor and outdoor allowed us to survey the preferred feeding patterns of mosquitoes while the human behavior data collected through the household surveys have allowed us to estimate actual patterns of human exposure. *Anopheles funestus* seems equally predisposed to bite indoor or outdoor, regardless of intervention treatments. However, *Anopheles gambiae* s.l exhibited its classically documented endophagic tendency, particularly in blocks where both insecticide treated nets and indoor residual spraying applied in combination. Data on the hourly mean catches of the malaria vectors indicates the peak biting of *An. gambiae* s.l was just after the average time that residents go to bed at approximately 20 hrs. Contrary to *An. gambiae*, the peak biting time of *An. funestus*, the predominant vector in the area was in the late hours of the night, well after people go to sleep in both intervention areas. The highest catch, regardless of the intervention treaments was between 4 and 5 hrs. Because residents typically go to indoors and into bed at 20 hrs and get up at 6hrs, rude estimates of the proportion of human exposure occuring indoors was high for both species, with mean values of 0.90 and 0.94 for *An. gambiae* and *An. funestus*, respectively, that are essentially unchanged by intervention status.

615

SEXUAL PERFORMANCE OF *AEDES ALBOPICTUS* (DIPTERA:CULICIDAE) MALES IN THE FRAME OF A STERILE INSECT TECHNIQUE PROGRAM

Sebastien Boyer

Institut de Recherche pour le Développement, Sainte Clotilde, Réunion

Aedes albopictus was described as a vector of at least 22 arboviruses. This species can transmit Alphavirus (Chikungunya, equine fever) and Flavivirus (4 dengue serotypes, yellow fever, west nile fever). This species is now considered as the most efficient vector of Chikungunya virus and second for the dengue. *Ae. albopictus* is well established in Asia (native area), North and South America, Africa, Europe and Australia. Its high vector competence is combined with an efficient spreading behaviour. Current mosquito control methods against this species consist of chemical or biological treatments. The major problem besides the persistence of insecticides in the field and their impact on non-targeted species is the rapid acquisition of insecticide resistance. In the context of an area-wide integrated vector management, the Sterile Insect Technique (SIT) could be suitable in La Reunion Island. The insularity creates a geographical isolation and the existence of cool and dry seasons bring a decrease in mosquito populations. The sexual performance of wild males of targeted populations needed to be investigated in a SIT strategy. The mating ability of males *Ae. albopictus* was tested with batches of females and different cage sizes under laboratory conditions (colony from Saint-Pierre, La Réunion). One male was able to inseminate an average of 9.5 females and filled an average of 15.5 spermathecal capsules. One male encaged with 2 females removed and replaced every 24 h for 12 days inseminated 5.3 females and filled 8.6 spermathecal capsules. One male with 10 females removed and replaced every 24 h for 14 days inseminated 8.6 females and filled 12 spermathecal capsules. In the last two experiments, a significant decrease of mated females was observed over time. The high number of mated females by one male is encouraging for a SIT control of mosquitoes. The duration of the male activity is also a good new, in spite of its decrease over time. These two results will be used to model the release of males Ae. alobpictus.

616

DEVELOPMENT OF A NEW BIOMARKER OF EXPOSURE TO *ANOPHELES* BITES BASED ON HUMAN ANTIBODY RESPONSES TO SALIVARY PROTEINS: FROM THE CONCEPT TO THE APPLICATIONS

Anne Poinsignon[1], Papa-Makhtar Drame[2], Badara Samb[3], Sylvie Cornelie[1], Cheikh Sow[4], Ibrahima Dia[5], Gilles Riveau[4], Lassana Konate[6], Cheikh Sokhna[7], **Franck J. Remoue**[2]

[1]IRD-UR016, Montpellier, France, [2]IRD-UR016/CREC, Cotonou, Benin, [3]University Cheikh Anta Diop, Dakar, Senegal, [4]NGO Espoir pour la Santé, Saint-Louis, Senegal, [5]Institut Pasteur, Dakar, Senegal, [6]Université Cheikh Anta Diop, Dakar, Senegal, [7]IRD-UMR198, Dakar, Senegal

Human antibody (Ab) IgG response to whole saliva of An. gambiae could be an epidemiological biomarker of exposure to An. gambiae bites. In the objective to increase the specificity to Anopheles exposure, the second step is to identify the salivary proteins i) specific to Anopheles genus and ii) antigenic in children exposed to malaria. First, the identification of immunogenic salivary proteins of An. gambiae by an immuno-proteomic approach was assessed. The second step was to design peptide sequences, from the selected An. gambiae gSG6 antigen using a bioinformatic approach, taking into consideration i) their potential antigenic properties and ii) the absence of cross-reactivity with other arthropods/organisms. The specific IgG Ab levels were then evaluated in Senegalese children in different context of malaria.

From five gSG6 peptides, one gSG6-P1 peptide presented all criteria to be an optimal candidate biomarker for evaluating exposure to An. gambiae bites. Indeed, in addition to high specificity to Anopheles genus, the anti-gSG6-P1 IgG level was associated with the intensity of exposure to An. gambiae bites. In addition, complementary studies indicated that gSG6-P1 represents a specific tool for detecting low exposure to An. gambiae and also one biomarker for evaluating the level of An. funestus bites. This new "salivary" biomarker of Anopheles exposure could be used as a geographic indicator for mapping the risk of malaria transmission and especially in low Anopheles density conditions, where entomological methods are limited in sensitivity (dry season, altitude or urban malaria). It could also represent a direct criterion of efficacy in the evaluation of anti-vector strategies.

617

THE EFFECTS OF SUBLETHAL PESTICIDE EXPOSURE ON VECTORIAL CAPACITY OF BITING INSECTS

Lee Cohnstaedt, Sandra Allan

United States Deparment of Agriculture, Gainesville, FL, United States

Vectorial capacity is the efficiency of an arthropod to transmit disease to susceptible hosts. The Ross-Macdonald mathematical model identifies the parameters determining disease transmission efficiency and can be used to determine the role of insecticide use in reducing vector-mediated disease transmission (as reported previously). The frequent contact of disease vectors with insecticides via aerial spraying, residual spraying in houses or on barriers, bed nets, and larval treatments will reduce disease transmission but the sublethal aspect of insecticide exposure may also increase the surviving insect's vectorial capacity and contribute to the

evolution of insecticide resistance. Sublethal exposures to insecticides has been known to change the biting activity, longevity, host seeking ability and possibly the intrinsic incubation period if the arthropod's physiology is changed. Sublethal effects must also be considered in disease resistance evolution because in addition to the altered vectorial capacity, the surviving mosquitoes will have a fitness advantage in the presence of pesticides. Therefore, sublethal effects of insecticides may be altering the vectorial capacity and increase the rate of insecticide resistance evolution.

618

FIRST DATA ON *AEDES ALBOPICTUS* DISPERSAL IN ITALY

Francesca Marini[1], Beniamino Caputo[1], Marco Pombi[1], Manuela Travaglio[2], Gianfranco Tarsitani[1], Fabrizio Montarsi[3], Andrea Drago[4], Alessandra della Torre[1]

[1]University of Rome "Sapienza", Rome, Italy, [2]University of Padua, Padua, Italy, [3]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padua, Italy, [4]Entostudio snc, Padua, Italy

We here present the results of *Aedes albopictus* Mark-Release-Recapture (MRR) experiments in which sticky-traps (STs) were used to collect released females. Three MRRs were carried out in a 250 m radius area within the campus of Sapienza University in Rome (RM, central Italy) in summer 2008, releasing about 500 blood-fed females and employing 55 STs (≈1 ST/3,600 m2) in each replicate. Other 3 MRRs were carried out in a 500 m radius area in a rural/periurban site in the Province of Padova (PD, northern Italy) in summer 2009, releasing about 1.000 blood-fed females and employing 94 STs (≈1 ST/8,400 m2) in each replicate. Recaptures were carried out for 16 days after releases. The recapture rates obtained ranged between 3.3 and 5.1% in RM and 3.4 and 13.4% in PD. Most re-captured females were collected at the gravid stage in the first 8 days after releases. This observation - coupled with the results of single oviposition experiments carried out in PD simultaneously to the releases - allows to conclude that our results mainly refer to the dispersal of females looking for an oviposition site after having completed a single gonotrophic cycle triggered by the blood-meal provided before releases. The females were mostly recaptured at 50-200 m and 0-150 m from the release sites in RM and PD, respectively. Single females flew up to 230 and 464 m in 4 days in RM and PD, respectively. In both sites the females reached the limit of the study areas, indicating that they may probably fly even further away. The cumulative mean distance travelled was 105, 121, 139 m in RM and 110, 77, 68 m in PD. These results will be discussed with reference to the ecological characteristics of the two study areas. These data represent the first evaluation of *Ae. albopictus* movements in an European area and are instrumental to plan control activities and to determine appropriate control limits necessary to interrupt pathogen transmission in case of possible arbovirus epidemics in Europe, such the Chikungunya outbreak occurred in northern Italy in 2007.

619

COMBINED SEWAGE OVERFLOW WATER QUALITY AND SEASONALITY EFFECTS ON *CULEX SPP* OVIPOSITION

An T. Nguyen, Luis F. Chaves, Uriel Kitron

Emory University, Atlanta, GA, United States

Larvae of *Culex quinquesfasciatus*, the main vector of West Nile virus in the southern US, are often found in combined sewage overflows (CSOs) in the city of Atlanta, GA. Semi-natural experiments were conducted to study the association of mosquito oviposition with fluctuating levels of chemical nutrients and seasonal weather variability in pools containing CSO water in Tanyard creek, Atlanta, GA. Semi-natural habitats were created in artificial isolated pools to compare oviposition frequency and intensity in protein-enriched CSO water with pools containing unenhanced CSO water. Water nutrients, dissolved organic N and P, in the isolated pools and the main creek, and oviposition rates in the isolated pools were compared over time. The addition of nutrients to these systems increased organic matter concentration and the oviposition rate. Water temperature and relative humidity changes in the environment had a direct impact on the number of oviposited egg rafts. These results are relevant to understand the spatial and temporal abundance of mosquito vectors and West Nile Virus transmission risk in urban settings.

620

A "CONSUMER PRODUCT" STUDY FOR USE OF INSECTICIDE TREATED CURTAINS TO REDUCE MOSQUITOES AND DENGUE INFECTIONS IN HOMES

María Alba Loroño-Pino[1], Julián García-Rejón[1], José Arturo Farfán-Ale[1], Arturo Losoya[2], Lyla Aguilar[2], José Trapaga-Barrientos[2], Saul Lozano-Fuentes[3], Lars Eisen[3], **Barry J. Beaty**[3]

[1]Universidad Autónoma de Yucatán, Merida, Mexico, [2]Bayer Environmental Science - Bayer de Mexico, Mexico City, Mexico, [3]Colorado State University, Fort Collins, CO, United States

Novel control strategies are essential to reduce vector-borne diseases. Empowering individual home owners to complement and enhance governmental control efforts is an attractive and innovative approach for vector control. A "Casa Segura" study was conducted in Merida, Mexico to determine if individual home owners could utilize insecticide treated curtains (ITCs), provided by Bayer de Mexico/Acytex Internacional, to reduce the number of vector mosquitoes (*Aedes aegypti*) and the risk for becoming infected with dengue virus in their own homes. The study included 411 homes and 2,042 participants. Paired homes were randomly selected to receive ITCs or non-treated curtains (NTCs). They were located 80-100 m apart (*Ae. aegypti* rarely disperses > 50 m in urban settings) to prevent any spill over effect of the ITCs. Epidemiological outcomes (including dengue virus infection and seroconversion) and entomological outcomes (including *Ae. aegypti* and *Culex quinquefasciatus* abundance in the homes) were monitored. Further, ITC killing efficacy over time was characterized in bioassays (WHO cone assay), and "consumer" acceptance, usage and satisfaction with the ITC product was determined. Preliminary results indicate a reduction in dengue infections in participants in homes with ITCs versus NTCs. Reductions in *Ae. aegypti* and *Cx. quinquefasciatus* abundance were initially detected in ITC homes, and other entomological outcome trends, e.g., presence of dengue virus infected *Ae. aegypti* females, also suggest a protective effect of the ITCs. The great majority of infected mosquitoes were collected from bedrooms. Finally, social scientist interviews conducted as part of the study revealed extensive use of aerosol, mosquito coil, and insecticide emanators in households in Merida to reduce indoor mosquito biting.

621

EVALUATION OF ZEROVECTOR® DURABLE LINING (DL) - RESTING AND IRRITANCY PATTERNS OF *AEDES AEGYPTI* UNDER VARYING SURFACE AREA COVERAGE

Joseph Wagman, Luana Arce, Pankhil Shah, Truc Vy Nguyen, Tarra Foggie, John Grieco, Nicole Achee

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

Maximum success from a particular vector control strategy results from optimizing the intervention. Such outcomes depend on characterizing species-specific behavioral responses of vectors against the product under evaluation. Recently, a *D*urable *Activated R*esidual *T*extiles (DART) product has been developed to replace the traditional indoor residual spray (IRS) strategy. The function of this durable-lining (DL) material is to transfer toxic doses of deltamethrin to vectors resting on the surface of the material thereby reducing overall populations and biting pressures to human hosts. The DL is fixed to the interior wall surfaces of homes in malaria endemic areas. As house dimensions vary, there are areas of the wall that are left exposed (i.e., no DL) thereby creating "safe-sites" where vectors may rest without making contact with chemical. The contribution of these safe-sites to overall product success - mortality and/or escape prior to biting was evaluated using laboratory methodologies. We present results

describing the resting preference, knock down (KD) and 24 h mortality responses of unfed *Aedes aegypti* females (THAI strain) exposed to varying surface area coverage of DL (100, 75, 50 and 25%) under laboratory conditions. In addition, rates of assay escape were quantified to determine time (i.e., probability of man-vector contact under field conditions) and density (i.e., total reduction of potential biting vectors) responses to DL material. For all assays in which treated DL was applied, there was less resting response overall - even on safe sites (i.e., metal surfaces) within the assay device- and significant increases in the proportion of test cohorts flying and exhibiting KD compared to matched controls. This indicates an agitation response from the chemical active that was true even at a 25% coverage ratio. When test cohorts were evaluated for escape response in subsequent movement assays, there was significant increase in percent escape compared to matched control assays at the 25, 50 and 75% DL coverage ratios after correcting for movement in control tests. Combined, these results suggest minimal negative effects of safe sites to the overall efficacy goals of the DL product. Similar studies will be repeated against blood-fed *Ae. aegypti* test cohorts in preparation for field validation.

622

SPATIAL SCALE OF DENGUE VIRUS TRANSMISSION IN IQUITOS, PERU

Kelly A. Liebman[1], Steven T. Stoddard[1], Brett M. Forshey[2], Amy C. Morrison[1], Tadeusz Kochel[2], Thomas W. Scott[1]

[1]University of California, Davis, Davis, CA, United States, [2]United States Navy Medical Research Center Detachment, Lima, Peru

Understanding spatial patterns of dengue virus (DENV) transmission is critical for the design of improved surveillance and control strategies. Although clinically apparent DENV infections are known to occur in households with similar onset of illness, a key unanswered question is at what scale transmission occurs beyond the home. Mosquito mark-release-recapture studies indicate that the DENV vector *Aedes aegypti* typically disperses only short distances (<100 m), leading to the prediction that cases will cluster within a 100 m radius. We carried out Bernoulli spatial analyses of DENV infections in a prospective longitudinal cohort of >4,000 people from 1999-2005 in Iquitos, Peru. Infection status was determined by seroconversion (neutralizing antibody) in paired blood draws taken every 6 months. Date of infection was assigned as the mid-date between paired samples, and each year was divided into 3 distinct seasonal periods of DENV transmission (Jan 1 - Apr 30, May 1 - Aug 31, Sept 1 - Dec 31). We identified serotype-specific spatial clusters at maximum radii of 100, 300, 600 and 900 meters, during each trimester, for all DENV serotypes circulating in Iquitos [5 DENV-1 clusters (1999, $p \leq 0.001$; 2002, $p=.008$), 1 DENV-2 (2000, $p=0.025$), and 15 DENV-3, beginning in 2001 when DENV-3 was first introduced (2001, $p<0.022$; 2002, $p<0.05$; 2003, $p<0.026$; 2004, $p=0.003$)]. The number of cases per cluster ranged from 3 to 39 with radii of 0 - 740 meters. Our results indicate that (1) interventions need to extend beyond a person's home to substantially reduce their risk of infection, (2) human movement beyond the flight range of *Ae. aegypti* is important for defining the spatial scale of DENV transmission, and, (3) if clusters of cases can be operationally and cost effectively identified, spatially targeted intervention strategies should be considered as a cost-effective way to prevent disease.

623

AN EVALUATION OF NON-TOXIC APPROACHES FOR DENGUE VECTOR CONTROL

Hortance Manda, Luana Arce, Pankhil Shah, Nicholas Martin, Truc Vy Nguyen, Tarra Foggie, John Grieco, Nicole Achee

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

Research shows that killing insects through direct chemical means may not be necessary for effective vector control. Other approaches to reduce man-vector contact at the house level exist and might be sufficient. This includes initiating a spatial repellent effect, preventing house entry; and/or contact irritant effect, causing an escape response prior to mosquitoes biting humans indoors. Such non-toxic approaches are currently being evaluated in this research project as proof-of-principle for a Push-Pull strategy for *Ae. aegypti* control. We are targeting preferred house entry portals and/or indoor resting sites to make them unsuitable, using minimum effective dose at minimum surface area coverage to reduce densities of mosquitoes inside homes, a site of disease transmission. A component to the overall success of the program is the ability to correlate chemical concentration in the surrounding air space of a treatment source with vector behavior over distance. This quantification will be used to describe behavioral thresholds of non-toxic control strategies and help to clarify the mode of action of target chemicals. The overall goal is to drive the development of innovative and cost-effective vector control strategies. This study reports on the contact irritancy and spatial repellency responses of two geographically distinct female *Ae. aegypti* strains (Thailand and Peru). Entry (i.e., repellency), escape (i.e., irritancy), and mortality rates were quantified in response to different doses of chemical and surface area coverage of standard vector control compounds under laboratory conditions. Air space at various distances away from the treatment source was sampled for chemical, and its concentrations quantified and correlated to the entrance and exit behavior of test mosquito populations. Experiments were also validated under field conditions using experimental huts. Results of this study will guide the development of air sample testing protocols as it relates to vector ecology and ultimately guide the optimal conditions for a Push-Pull strategy trial.

624

EVALUATION OF THE SPECIES COMPOSITION AND RELATED ECOLOGY OF BLACK FLIES (DIPTERA: SIMULIDAE) FROM BELIZE, CENTRAL AMERICA

Cecilia L. Coscaron-Arias[1], Nicole Achee[1], Russell King[2], Ireneo Briceno[2], John Grieco[1]

[1]Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [2]Ministry of Health, Belmopan, Belize

Across the Americas, over 500,000 people live in areas at risk for the transmission of Onchocerciasis and over 180,000 are infected in endemic areas. It is well documented that this disease is transmitted by black flies in the family Simulidae. The members of this family occupy a diverse range of environments and habitats including man-made sites such as dams and irrigation canals. Black flies from Guatemala and Mexico have been studied extensively due to the endemic foci of onchocerciasis found in these countries. However, the simulids from Belize, a neighboring country, are not well known and current information for the country only exists for the most southern regions. Information that does exist suggests that five of the thirteen species found in Belize are known to vector onchocerciasis. In addition, two other species are known to vector carate or mal de pinto and Venezuelan Equine Encephalitis. Due to the lack of information from this region of Central America with regard to the potential risk for the transmission of onchocerciasis, this study evaluated the species distribution and related ecology of black flies from Belize. The resulting data was incorporated into a GIS platform to display the areas at risk for the dominant vector species in the region. The resulting risk maps can be used to guide surveillance and control efforts in Belize.

625

CONTEXT-DEPENDENT OVIPOSITION STRATEGIES BY *AEDES AEGYPTI*

Jacklyn Wong, Yui Yin Chu, Steven T. Stoddard, Helvio Astete, Amy C. Morrison, Thomas W. Scott

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

Effectiveness of *Aedes aegypti* surveillance and control strategies depends on assumptions about oviposition behavior. Sensitivity of ovitraps for detecting *Ae. aegypti* presence and efficacy of lethal ovitraps for reducing

adult populations may be overestimated if skip oviposition occurs less frequently than anticipated. Effectiveness of targeted source reduction is based on the assumption that females egg-saturate all available oviposition sites. Removing a portion of containers must result in a net reduction in adult population because productivity in remaining containers is already at maximum (as reported previously). In this study, we tested the hypothesis that *Ae. aegypti* egg allocation strategies are context-dependent. We predicted that given a choice between large containers with organic debris and small containers with clean water individual females would concentrate their eggs in one large container, leaving many small containers unoccupied. If large containers were removed, we predicted that females would switch to an alternate strategy, distributing their eggs widely among several small containers. We released into a field enclosure 12- 20 F1 females that had mated with known males in the laboratory and collected their eggs daily from eight potential oviposition containers. Our experiment was repeated three times with both large and small containers available, and twice with only small containers present. Using 10 microsatellite markers, we are assigning progeny to parental pairs (Probmax version 1.2, as reported previously) and tracking when and where each female laid her eggs over 7-10 days after release. We expect that removal of the most productive containers will not lead to a simple, proportional reduction in mosquito population size. Detailed studies of where wild females allocate their eggs and how they respond to targeted control measures will test fundamental assumptions of this frequently recommended control strategy and provide insights into improved dengue surveillance and control.

626

INSECTICIDE SUSCEPTIBILITY OF *PHLEBOTOMUS PAPATASI AND P, SERGENTI* (DIPTERA: PSYCHODIDAE) FROM TWO GEOGRAPHICAL REGIONS OF EGYPT USING CDC BOTTLE AND MICROPLATE ASSAYS

Abdelbaset B. Zayed[1], Daniel E. Szumlas[1], Hanafi A. Hanafi[1], David F. Hoel[1], Barry D. Furman[1], Peter J. Obenauer[1], Hanaa M. Mahmoud[2], Reham M. Abolwafa[1]

[1]United States Naval Medical Research Unit Number Three, Cairo, Egypt, [2]Faculty of Science, Al-Azhar University (Girls Branch), Cairo, Egypt

Phlebotomine sand flies vector cutaneous leishmaniasis (CL), is a serious disease infecting 1,500,000 persons per year over throughout the world. According to WHO 2008, the Middle East harbors about 15% of the global leishmaniasis burden. In Egypt, the North Sinai is the most CL epidemic area. Evaluation of insecticide resistance is an important component in any integrated vector control program. Field trials were conducted from two regions in Egypt to evaluate insecticide resistance from two populations of *Phlebotomus papatasi* and *P. sergenti*. Insecticide susceptibility of both species was observed in samples collected from North Sinai (desert area, 450 Km north east Cairo) and Aswan (agriculture area, 950 Km south Cairo) governorates. Insecticide resistance was detected using the CDC bottle assay and confirmed by biochemical mircoplate assays. First generation of sand flies from both species were exposed to seven insecticides at the following doses (µg/ bottle): permethrin (150), resmethrin (250), lambdacyhalothrin (3), deltamethrin (5), cypermethrin (5), malathion (150) and fenitrothion (120). Time-dose mortality curves demonstrated that, *P. papatasi* from Sinai were more susceptible than those collected from Aswan to lambdacyhalothrin, deltamethrin, cypermethrin, malathion and fenitrothion. While both populations were resistant to resmethrin and permethrin, *P. sergenti* demonstrated low resistance to deltamethrin and lambdacyhalothrin, cypermethrin and resmethrin. *P. papatasi* population collected from Aswan show high level of enzymes activity in AChE, insensitive AChE, EST, GST and Oxidase compared with the laboratory population, while Sinai populations demonstrated a high level of EST, EChE and Oxidase. No increased levels in detoxified enzymes were observed in the *P. sergenti* population collected from Sinai. These results confirmed insecticide susceptibility for this population.

627

SURVEY OF RICKETTSIAL VECTORS AND RESERVOIR HOSTS IN MILITARY AREAS OF OPERATION (AOS) ALONG NORTHERN THAI-MYANMAR AND THAI-CAMBODIA BORDERS

Pimmada Sakpaisal[1], Toon Ruang-areerate[1], Narupon Kuttasingkee[1], Nopadol Sangjun[1], Kiatisak Somsri[1], Jittasak Koewsathit[2], Khwananong Youngpakool[1], Pradith Kaewsatien[1], Chaiya Chanchu[1], **Jariyanart Gaywee**[3]

[1]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [2]Faculty of Sciences, Chulalongkorn University, Bangkok, Thailand, [3]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

Rickettsioses have been reported in military troops deployed to military areas of operation (AOs) along northern Thai-Myanmar and Thai-Cambodia borders. Causative agents, rickettsiae are intracellular bacteria maintained in nature via small mammalian hosts and blood sucking arthropod vectors. To better understand how these pathogens are transmitted to humans, we investigated these AOs for the presence of rickettsial reservoir hosts and vectors. Wild rodents were captured and there ectoparasites were collected. We also collected ectoparasitic arthropods from livestock, pet animals and humans in these AOs. *Rickettsia-Orientia* duplex nested PCR as well as sequencing were utilized to detect and identify rickettsial agents. From April 2008 to December 2009, we have collected and tested 133 clones of arthropods including twelve species of fleas, lice, and ticks from different hosts and locations. Rickettsial genes were detected in 80.7% and 74.8% of arthropods from Northern Thai-Myanmar and Thai-Cambodia border areas, respectively. Species of pathogenic rickettsiae identified by 17 kDa sequence analysis were *Rickettsia japonica*, *R. rickettsii* and *R. massiliae*. Those rickettsiae were detected in ticks (*Dermacentor* sp., *Rhipicephalus* sp. and *Haemaphysalis* sp.) collected from humans and pet dogs. Spotted fever group *Rickettsia* sp. similar to *Rickettsia* sp. Cf 1, Cf 5 and SE 313 were also detected in fleas (*Ctenocephalides canis*, *C. felis* and *Echidnophapga gallinacean*) and lice (*Liperus caponis*, *Menopon gallinae* and *Haematopinus asini*) collected from dogs, cats, cattle and chickens. *Orientia tsutsugamushi* DNA was not detected. Using ISE6 tick cell culture, 2 rickettsial isolates were obtained from *Dermacentor* and *Haemaphysalis* ticks collected from dogs in AOs along the Thai-Cambodia border. Specific species identification of theses isolates is ongoing. These findings indicate these AOs are endemic foci for rickettsioses. This information is crucial to establish an effective disease prevention and control strategy specific to such areas.

628

WOLBACHIA INFECTION AND *PLASMODIUM* DEVELOPMENT IN *ANOPHELES GAMBIAE*

Grant L. Hughes, Jason L. Rasgon

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

Recently, *Wolbachia pipientis,* an endosymbiotic bacterium of insects and nematodes, has been shown to interfere with pathogen development in stably transinfected *Aedes aegypti,* possibly due to stimulation of the mosquito innate immune system. Moreover, it can confer a fitness advantage to the host in naturally infected *Drosophila* when challenged with a virus. In these systems, *Wolbachia* acts against a broad range of pathogens, including viruses, bacteria, nematodes and *Plasmodium*. *Wolbachia,* which has not been detected in any species of *Anopheles,* has never been transinfection into this genus, despite numerous attempts to establish a stable line. However, transient somatic infections in *Anopheles* can be established. Here, we investigate the effect of somatic infections with multiple *Wolbachia* strains on *Plasmodium* development in *Anopheles gambiae*. In somatically infected *Anopheles*, *Wolbachia* is ubiquitously disseminated throughout the mosquitoes, however is noticeably absent

from the ovaries. After adult microinjection, normalized *Wolbachia* titers initially decrease, presumably due to the host immune response clearing the bacteria, but then increase dramatically approximately 2 weeks post-injection. Microarray analysis of *Wolbachia*-infected cell lines identified a suite of regulated host genes, with a range of immune-related genes both up and down regulated. In somatically infected wMelpop mosquitoes, qPCR identified down regulation of immune genes 15 days post injection. The wAlbB *Wolbachia* strain (from *Aedes albopictus*) induced both up and down regulation of immune genes. Our initial data indicates that somatically-infected *An. gambiae* do not have resistance to *Plasmodium falciparum* or *Plasmodium berghei*, suggesting that the interplay between *Wolbachia* and *Anopheles* differs from previously observed interactions. These observations may be related to why the *Anopheles* genus is uninfected in nature, with implications for developing a stably-infected *Anopheles* line.

629

DIPETALODIPIN, A NOVEL SALIVARY PLATELET AGGREGATION INHIBITOR THAT DISPLAYS HIGH-AFFINITY BINDING TO TXA_2

Teresa C. Assumpção, Patricia H. Alvarenga, José M. Ribeiro, John F. Andersen, Ivo M. Francischetti

National Institutes of Health, Rockville, MD, United States

Dipetalodipin (DPTL) is an 18 kDa protein from the salivary gland of hematophagous triatominae *Dipetalogaster maximus*. It belongs to the lipocalin superfamily of proteins and shows sequence similarity to pallidipin, a lipocalin from *Triatoma pallidipennis*, claimed as a specific inhibitor of collagen-induced platelet aggregation. Recombinant DPTL was found to inhibit platelet aggregation triggered by collagen, without interfering with neither GPVI nor integrin $\alpha2\beta1$-mediated platelet adhesion. DPTL also prevented platelet responses induced by U-46619 and arachidonic acid, without affecting aggregation induced by ADP, convulxin, PMA, and by ristocetin. This inhibitory profile suggested that DPTL targets a secondary mediator released from activated platelets. An assay based on incubation of DPTL with small molecules (e.g. prostaglandins, leukotrienes and biogenic amines) followed by gel-filtration chromatography, and mass spectrometry was optimized in an attempt to identified DPTL-bound ligand(s). Results indicated the presence of a ligand with molecular mass of 351-352 which is compatible with prostaglandins. Identification of the compound was attained by isothermal titration calorimetry which demonstrated that DPTL binds with high affinity to cTXA2, TXA2-mimetic (U-46619) and structurally related prostaglandins such as PGH2, PGF2α, and PGD2. Consistent with its binding properties, DPTL prevents rat aorta contraction stimulated by U-46619, and its effect was abolished when collagen-induced platelet aggregation was attenuated with SQ29,548, an antagonist of TXA2 receptor. A 3D model for DPTL is presented where the putative binding site is indicated. Our results demonstrate that Dipetalodipin, and presumably pallidipin, are platelet aggregation inhibitors with unique specificity to TXA2.

630

PERCEIVED UTILITY OF TICK IDENTIFICATION FOR CLINICAL MANAGEMENT OF TICK-BORNE DISEASES

Rachel E. Tsui[1], Jon E. Rosenblatt[2], Bobbi S. Pritt[2]

[1]St. George's University of London, London, United Kingdom, [2]Mayo Clinic, Rochester, MN, United States

Ticks are globally important ectoparasites that transmit multiple pathogens. They are routinely submitted to many clinical parasitology labs for identification although the extent of characterization is highly variable and the clinical relevance is not well described. The goal of this study was to define the role that tick characterization plays in clinical management, and determine which features are perceived as being clinically important. A 5-question email survey was sent to all Minnesota-based Mayo Clinic primary care physicians and nurse practitioners. Information regarding the use of lab tick identification services and disease prophylaxis was obtained. Of the 1008 surveyed, 153 (15.2%) responded. 74 reported seeing ticks in their practice, of which 29% would submit a tick to the lab for identification when removed in the office, and 31% would submit a tick if brought in by the patient. 24% would only submit select ticks based on patient symptoms or request, and 16% would not submit a tick to the lab under any circumstances. 36% would administer prophylaxis for tick-borne disease based solely on the presence of a tick, regardless of lab identification, whereas 20% would only administer prophylaxis if the laboratory identified the tick as a potential disease vector. The remaining 44% would base prophylaxis decisions on several factors. Of the information provided by the lab, species, degree of engorgement, presence/absence of mouthparts, gender, and life cycle stage were perceived as useful by 32, 11, 9, 6 and 6 respondents, respectively. PCR for tick-borne pathogens performed on the tick was perceived to be useful by 43 respondents. 60% of respondents would routinely submit ticks to the lab for identification if they were found on or by the patient, but only 20% required identification of a known disease vector before providing prophylaxis. Tick speciation was perceived as being the most useful morphologic feature although more respondents considered PCR for pathogens most useful, even though this is not supported by clinical recommendations.

631

SARCOPTES SCABIEI EXTRACT INFLUENCES EXPRESSION OF NFκB (NUCLEAR TRANSCRIPTION FACTOR KAPPA B) THAT CONTROLS EXPRESSION OF IL-8 GENES

B. Laurel Elder, Larry G. Arlian, Marjorie S. Morgan

Wright State University, Dayton, OH, United States

Previous experiments in our laboratory have indicated that exposure of human dermal microvascular endothelial cells to an extract made from *Sarcoptes scabiei* mite bodies results in a decrease in detectable interleukin-8 in the endothelial cell supernatant. We attempted to elucidate the mechanism that might be involved in the suppression of this IL-8 secretion. Several cellular pathways are proposed that lead to IL-8 secretion but in the final stage all utilize up- or down-regulation of nuclear transcription factor kappa B (NFκB) to control expression of IL-8 genes. NFκB is activated and able to enter the nucleus when its inhibitor IκB is phosphorylated and degraded. We hypothesized that scabies extract might inhibit the phosphorylation of IκB resulting in suppression of IL-8 genes. We found that TNFα-stimulated endothelial cells increased expression of nuclear NFκB as expected (positive control). Cells stimulated with scabies extract decreased NFκB expression as postulated but also expressed some other low molecular weight proteins that were bound by antibody to NFκB. Cells stimulated with mite extract expressed more cytoplasmic IκB (the NFκB inhibitor) implying that a component of the extract may be interfering with the phosphorylation of IκB. The meaning of these results is not yet clear but the data suggest that some component or components in scabies extract may act at this level of the pathway to influence the secretion of IL-8.

632

NATURAL INFECTION OF *LEISHMANIA* SPECIES IN *SERGENTOMYIA INGRAMI* AND *S. HAMONI* IN AN OUTBREAK AREA IN THE HO DISTRICT OF GHANA

Chukwunonso O. Nzelu[1], Shirley C. Odoom[1], Kwame Desewu[2], Naiki Puplampu[1], Karl C. Kronmann[3], Anita Ghansah[1], Michael D. Wilson[1], Daniel A. Boakye[1], Delphina Gomez[4]

[1]Noguchi Memorial Institute for Medical Research, Legon, Accra, Ghana, [2]Ghana Health Service, Accra, Ghana, [3]United States Naval Medical Research Unit 3, Ghana Detachment, Legon, Accra, Ghana, [4]Atomic Energy Commission, Accra, Ghana

Following the leishmaniasis outbreak in the Ho district of Ghana in 2004, studies have been going on to determine the causative parasite, the reservoir hosts and the sandfly vector species. The parasite species have been reported to be *Leishmania major* and a yet to be characterized species indicating a complex epidemiology for this outbreak than anticipated. Sandfly samples collected from 2005 to 2008 also recorded almost 100% Sergentomyia species. In 2009 blood meal analysis from blood-fed flies collected in human habitats indicated anthropophily of 3 *Sergentomyia* species: *S. ingrami, S. africana africana* and *S. simillima*. As a follow up on that study, we examined non-blood fed populations of these 3 species as well as others collected at the same time as the blood-fed ones for the presence of *Leishmania* parasites. A total of 951 sandflies belonging to 9 different *Sergentomyia* species were identified based on morphology. The major ones were *S. africana africana* (30.0 %); *S. ingrami* (22.7 %); *S. dissimillima* (20.8 %); *S. simillima* (19.6 %), and *S. hamoni* (5.9 %). Female sandflies were pooled in groups of 10 for DNA extraction and PCR for infection with *Leishmania* parasites. Thirty-four pools composed 7 *S. africana africana*, 8 *S. ingrami*, 7 *S. dissimillima*, 6 *S. simillima* and 6 *S. hamoni* were obtained. Two of the *S. ingrami* pools and one of *the S, hamoni* pools were positive. These results show for the first time the natural infection of *Sergentomyia* species (*S. ingrami* and *S. hamoni*) with *Leishmania* parasite in Ghana, and builds on earlier data indicating the possibility of *Sergentomyia* species as vectors of cutaneous leishmaniasis in Ghana similar to the suspicion of *S. ingrami* as potential vectors of *L. major* in Kenya.

633

WOLBACHIA INFECTION DYNAMICS IN TSETSE FLY POPULATIONS ACROSS UGANDA

Uzma Alam[1], **Corey Brelsfoard**[1], Oleg Kruglov[1], Yineng Wu[1], Loyce Okedi[2], Serap Aksoy[1]

[1]Yale University, New Haven, CT, United States, [2]National Livestock Resources Research Institute, Tororo, Uganda

Tsetse flies (*Diptera Glossinidae*) are the single vector for the protozoan parasite African trypanosomes, which are the causative agents of Human African trypanosomiasis (HAT), or sleeping sickness in humans and nagana (AAT) in animals. The incidence of HAT is geographically separated along the line of the Great Rift Valley with *Trypanosoma brucei rhodesiense (Tbr)* present in East and Southern Africa and *Trypanosoma brucei gambiense (Tbg)* in West and Central Africa. The disease epidemiology in Uganda is unique considering that it is the only African country known to have both *Tbr* and *Tbg*. *Glossina fuscipes fuscipes* transmits both parasite species, with *Tbg* transmission occurring in the northwest region and *Tbr* in the southeast limited to areas close to the shores of Lake Victoria. This segregation had held in place despite the movement of cattle and people. Recently the presence of *Tbr* in the mid-western part of Uganda has been confirmed in a three patients. This merger of the two disease belts is feared and stands to create a major public health crisis given the differences in diagnosis and treatment options. Based on mtDNA haplotypes, we have shown that tsetse populations across Uganda are highly differentiated. Based on this finding we hypothesized that a contributing factor to the high levels of differentiation observed could be circulating *Wolbachia* genotypes in the different populations. We used the *Wolbachia groEl* gene to screen *G. f. fuscipes* populations across Uganda for the presence of parasitic infections and *Wolbachia* infection types. Using a multiple locus sequence typing (MLST) approach we demonstrate that natural tsetse populations are infected with multiple *Wolbachia* types. We present a spatial and temporal map of *Wolbachia* infection dynamics and discuss their potential impact on tsetse population structures observed.

634

DETECTION OF *BLA*$_{CTX\text{-}M\text{-}15}$ EXTENDED-SPECTRUM-LACTAMASE GENES IN *E. COLI* FROM HOSPITAL PATIENTS IN NIGERIA

Adekunle O. Olowe[1], Lothar Wieler[2], Mirjam Brobbel[2], Antina lube-Becker[2], A. Fruth[3]

[1]Ladoke Akintola University of Technology, Osogbo, Osun State, Nigeria, [2]Institutes for Microbiology and Epizootics, Freie University, Berlin, Germany, [3]Robert Koch Institute, Wernigerode Branch, Germany, Wernigerode, Germany

The aim of this study was to investigate the occurrence and relatedness of CTX-M type extended spectrum -Lactamases (ESBL) in *Escherichia coli* isolates from patients of a Nigerian Hospital. The study included 116 *E. coli* isolated from inpatients and outpatients from January 2006 to January 2007 at the Ladoke Akintola University Teaching Hospital in Osogbo, Nigeria. The phenotypical confirmation test revealed 12 ESBL positive isolates, which were investigated for the presence of *bla*CTX-M genes. Nine of these *E. coli* contained *bla*CTX-M group 1 genes and additionally harbored *bla*TEM and *bla*OXA group 1 genes. Pulsed field gel electrophoresis (PFGE) of these 9 strains revealed 6 clonal groups, as four of the isolates revealed identical PFGE patterns while the other five showed no relatedness. Sequencing of the *bla*CTX-M gene of one isolate from each clonal group always identified CTX-M 15. At present there are no published data about the genetic background of ESBL-producing *E. coli* in Nigeria. To our knowledge, this is the first report of *E. coli* carrying *bla*CTX-M-15, *bla*TEM, and *bla*OXA genes in Nigeria. Further studies are ongoing on blaCTX-M enzymes situation in zoonotic isolates as it relates to man.

635

IN VIVO EFFICACY OF CIPROFLOXACIN, CEFTRIAXONE AND DOXYCYCLINE ALONE AND IN THEIR COMBINATION AGAINST *VIBRIO VULNIFICUS* INFECTION

Ganesh-Prasad Neupane, Dong-Min Kim

Division of Infectious Diseases, Department of Internal Medicine, Chosun University, College of Medicine, Kwangju, Republic of Korea

Ciprofloxacin, doxycycline and ceftriaxone; highly recommended drugs for the treatment of invasive *Vibrio vulnificus* infection were evaluated in high dose bacteria inoculated (1 × 108 CFU) iron loaded ICR mice (n=10). Mouse survival rate was calculated by Kaplan- Meier survival curve after monitoring for 48-h, and a highly sensitive quantitative PCR assay was performed to evaluate the number of DNA copies from viable bacterial cell in mouse blood drawn immediately after died or moribund by cardiac puncture. DNA binding dye Ethidium bromide monoazide (EMA) was used to differentiate viable and dead cells. The efficacies of ciprofloxacin, cefotaxime and doxycycline (oral and ip) route was compared with different combination therapy. The number of DNA copies was found decreased with increase of survival time of mice (1 × 104 - 1 × 105 CFU at 24h in dead mice). However, the number of DNA copies was 1 × 102 CFU or below than this level at 24 h survival mice in either monotherapy or combination therapy. Ciprofloxacin was the most effective drugs for monotherapy with high survival rate of 25 % at 48h. In combination therapy, a single dose doxycycline (i.p.) plus ceftriaxone was sufficient to reduce the mortality by 50 % in high dose *Vibrio* inoculated iron loaded mice in contrast to survival rate of 40 % in doxycycline oral plus ciprofloxacin treatment groups at least in our mouse

model infection. Furthermore, out of three combinations only doxycycline i.p. plus ceftriaxone showed significant ($P < 0.05$) versus doxycycline oral plus ceftriaxone treated group. Similarly, doxycycline i.p. plus ciprofloxacin ($P = 0.056$), and ciprofloxacin plus ceftriaxone ($P = 0.9$) did not showed significant result versus doxycycline oral plus ceftriaxone. Hence, in conclusion, cefotaxime plus doxycycline (i.p.) combination therapy might be the best treatment option among monotherapy or other combination therapy for lowering the high mortality rate of *Vibrio vulnificus* infection.

636

ANTIBIOTIC SUSCEPTIBILITY OF ENTEROBACTERIACEAE ISOLATED FROM COSTEÑO ARTISAN CHEESE SOLD IN MONTERIA DEPARTMENT OF CORDOBA, 2009

Linda M. Chams, Alberto Mestra, Maria F. Yasnot

Grupo de Investigaciones Microbiologicas y Biomédicas de Córdoba, Universidad de Córdoba, Monteria, Colombia

Infections caused by *Enterobacteriaceae* with multiple resistance to antimicrobial drugs, are a serious problem which must be faced daily by Physician, Veterinarians and Microbiologists. The use of these antibiotics, often indiscriminately, brings with it the selection of resistant mutants. The incidence of different species as well as the level of resistance to antibiotics most commonly used is a phenomenon with its own characteristics so it is essential to monitoring the evolution of resistance in microorganisms. The objective of this study was to determine the susceptibility of *Enterobacteriaceae* isolated from costeño artisanal cheese, to commonly used antibiotics in the treatment of humans and cattle. Descriptive exploratory study was conducted in 25 retail store in the city of Monteria, capital of the department of Cordoba, Colombia, duly registered with the Secretariat of Health of the department of Cordoba in 2009. 3 samples were taken from store, one every two months for a total of 75 samples, which were evaluated on color, smell and consistency. To isolate and to identify Enterobacteriaceae producing antibiotic resistence, we used the method of the FDA and used TSI, LIA, CITRATE, MR / VP, UREA and SIM. Serology was performed with the Kauffman-White scheme and sensitivity by the agar diffusion method under the international standards issued by the CLSI, 2008. The results were analyzed with the statistical software EXCEL. *E. coli* were isolated in the 75 samples (100%) tested, 7 of the 75 samples (9,3%) showed *Salmonella spp.*, and in 68 of the 75 samples (90,7%) analyzed was isolated Citrobacter spp. All strains isolated were resistant to amoxycillin for 100%, tetracycline 87.5%, Gentamicin and Chloramphenicol 70% c/u and 62.5% to amikacin. In conclusion, given that the costeño cheese is a food prepared from raw milk, a fact which get worse by deficiencies in sanitation and hygiene of outlets that sell this high regional consumption product, the high resistance percentage of Enterobacteriaceae isolated in these product to antibiotics is a concern, and turn on the alarms on their indiscriminate use, a fact that may cause the emergence of multiresistant strains to transfer this resistance to commensal and to pathogenic bacteria in food and bacteria belonging to the gastrointestinal flora of the consumer, making a serious public health problem.

637

TYPHOID FEVER WITH NEUROLOGIC FINDINGS -- MALAWI-MOZAMBIQUE BORDER, 2009

Emily C. Lutterloh[1], Andrew Likaka[2], James Sejvar[3], Tadala Khaila[2], Jeremias Naiene[4], Eric Mintz[3], Robert Manda[2], Michael Humphrys[3], Abel Phiri[2], Rudia Lungu[2], James Kaphiyo[2], Deborah Talkington[3], Kevin Joyce[3], Beth Tippett Barr[3], Austin Demby[3], Linda Capewell[3], Sara A. Lowther[3], David Townes[3], Kashmira Date[3], Yanique Redwood[3], Joshua Schier[3], Steve Monroe[3], Malawi-Mozambique Outbreak Investigation Team[3]

[1]New York Department of Health, New York, NY, United States, [2]Malawi Ministry of Health, Lilongwe, Malawi, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States, [4]Mozambique Ministry of Health, Maputo, Mozambique

Salmonella enterica serovar Typhi is the agent of typhoid fever, which often presents with fever and abdominal pain and is transmitted by the fecal-oral route. Annually, an estimated 22 million cases and 216,500 deaths occur worldwide. We investigated an outbreak of unexplained febrile illnesses with neurologic findings, determined to be typhoid fever, in villages along the Malawi-Mozambique border.

Methods: Ill persons were identified through Malawi Ministry of Health surveillance. We gathered demographic and clinical information on ill persons for March-November 2009 by interview, examination, and chart review. Classification as a suspect case required fever and ≥1 other finding (e.g. headache, abdominal pain); a probable case required fever and a positive rapid IgM antibody test for typhoid (TUBEX® TF); a confirmed case required isolation of Salmonella serovar Typhi from blood or stool. Isolates underwent antimicrobial susceptibility testing. Local springs used for drinking water were tested for total coliform bacteria and Escherichia coli with presence-absence broth. We identified 204 suspect, 47 probable, and 37 confirmed cases from 18 villages. Median age was 21 years (range: 1-81 years); 56% were female. Forty-three patients had neurologic signs including ataxia, hyperreflexia, and clonus. Of these 43 patients, 16 (80%) of 20 had positive rapid typhoid tests, and 4 (67%) of 6 blood cultures yielded Salmonella serovar Typhi. All 27 isolates that were tested were resistant to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole; 3 were also resistant to nalidixic acid. All three village springs tested were positive for total coliform bacteria and E. coli. In conclusion, the unusual neurologic manifestations of certain patients during this typhoid outbreak initially posed a diagnostic challenge. Rapid typhoid antibody testing in the field supported the diagnosis. Culture confirmation with antimicrobial susceptibility testing guided treatment. Recommended control measures include improvements in water quality, sanitation, and hygiene.

638

ANTIGEN SPECIFIC MEMORY B-CELL RESPONSES IN BANGLADESHI ADULTS AFTER ONE OR TWO DOSE OF ORAL CHOLERA VACCINATION, AND COMPARISON WITH NATURAL INFECTION

Mohammad Murshid Alam[1], Md. Asrafuzzaman Riyadh[1], Kaniz Fatema[1], Mohammad Arif Rahman[1], Nayeema Akhtar[1], Tanvir Ahmed[1], Mohiul Islam Chowdhury[1], Fahima Chowdhury[1], Stephen B. Calderwood[2], Jason B. Harris[2], Edward T. Ryan[2], Firdausi Qadri[1]

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

Natural infection with *Vibrio cholerae* O1 induces a robust systemic and mucosal immune responses that result in protective immunity against subsequent disease that lasts for at least 3-10 years. In contrast, protective immunity following vaccination with the whole cell killed oral cholera vaccine containing recombinant CtxB (Dukoral, Crucell, Sweden) is of shorter duration. The development of memory B cell responses, which may be important mediators of protective immunity, have not been

previously studied in vaccinees. In this study, we examined memory B cell responses in adult Bangladeshi individuals who received one (n=30) or two (n=30) doses of Dukoral, and we compared responses in 41 adult cholera patients. Vaccination induced vibriocidal antibody, and CtxB and lipopolysaccharide (LPS)-specific antibody responses in plasma within 3 days of the first dose of the vaccine ($P<0.001$) in both the groups of vaccinees. At 30 and 90 days after immunization, the responses (both magnitude and response rates) were comparable in both vaccine cohorts. Vaccinees developed significant CtxB-specific IgG and IgA memory B cell responses by day 30 post-immunization, and the CtxB-specific IgG memory B cell responses persisted for three months. The response to LPS was lower. In comparison to vaccinees, patients infected with wild type *V. cholerae* O1 mounted higher vibriocidal, CtxB and LPS-specific antibody responses at day 30. The memory B cell responses to CtxB were similar between patients and vaccinees; the LPS-specific responses were similar but lower in both groups as well. The vaccine induced an anamnestic response that was detected within 3 days, suggesting that protection can be induced very rapidly in a previously exposed population. Both a single and a two dose vaccine regimen resulted in a similar longevity of antibody and memory B cell responses. Thus, in settings where cholera is common, a single dose of the vaccine may induce sufficient immune responses to possibly confer protection.

639

EPIDEMIC CHOLERA IN KAKUMA REFUGEE CAMP, KENYA: THE IMPORTANCE OF SANITATION AND SOAP

Abdirahman Mahamud[1], Jamal Ahmed[1], Raymond Nyoka[1], Erick Auko[1], Vincet Kahi[2], James Ndirangu[2], Margaret Nguhi[2], John Burton Wagacha[3], Bosco Muhindo[3], Robert Breiman[1], Rachel Eidex[1]

[1]Centers for Disease Control and Prevention-Kenya, Nairobi, Kenya, [2]International Rescue Committee-Kenya, Nairobi, Kenya, [3]United Nations High Commission for Refugees, Nairobi, Kenya

Refugee camps are vulnerable to cholera outbreaks due to limited resources, poor sanitation and overcrowding. Kakuma Refugee Camp in Kenya has experienced recurrent cholera outbreaks. We report on findings from a cholera outbreak from September-December, 2009 with 224 cases and four deaths. We conducted a case control study in December 2009. Cases were identified by reviewing the hospital registry for patients meeting the WHO case definition for cholera. Two controls were selected per case; one was matched to the case based on area of the camp (the camp is separated into four areas) and age group (<5, 5-14, 15-24, and >25 years), and the second was an unmatched control selected by three-stage sampling method. A questionnaire focusing on potential risk factors was administered to cases and controls. A total of 93 cases, 93 matched and 126 unmatched controls, were enrolled into the study. In a multivariate model including cases and matched controls, washing hands with soap was protective against cholera (Adjusted Odds Ratio [AOR] =0.25; p<0.01) while presence of dirty water storage containers was a risk factor (AOR=4.4; p=0.03). In the multivariate model including cases and unmatched controls, using a latrine consistently was protective against cholera (AOR=0.13; p<0.01), whereas children not using a latrine (AOR=2.8; p=0.02) and living in Area A (AOR=10.23; p<0.0001) were risk factors. In conclusion, provision of soap, along with education on hand hygiene may be considered, as an affordable intervention to prevent cholera. Additional education may be helpful on importance of cleaning water storage containers, and latrine use. Areas with higher disease burden should be prioritized for these interventions.

640

BACTERIOLOGICAL AND PHYSICAL QUALITY OF LOCALLY PACKAGED DRINKING WATER IN KAMPALA CITY, UGANDA

Ali Halage, David Guwatudde, John Ssempebwa, David K. Ssemwanga

Makerere University College of Health Sciences, School of Public Health, Kampala, Uganda

Drinking water packaged in bottles and polythene bags has become a common consumer product in Kampala City, however, the quality of packaged water is unknown. The study aimed at assessing quality of locally packaged water sold for public consumption in Kampala city. We carried out a descriptive cross-sectional study, during January - March 2009. We collected 60 samples of bottled water from ten brands and 30 samples of sachet water from 15 brands. Bacteriological quality analysis used the membrane filtrate method with m lauryl sulphate broth as culture medium at Kasangati Public Health Laboratory. A quarter of all samples were sent to a reference laboratory (National Water and Sewerage Corporation) for validation. The samples were analyzed for total and faecal coliform organisms per 100 ml and reported in terms of cfu/100 ml. The sign test test and odds ratios were used to measure the difference in total coliforms between bottled and sacket water with the level of significance taken to be p<0.05.

Consumer perceptions towards packaged water were assessed from 423 respondents obtained by simple random sampling from 12 parishes in 3 divisions of Kampala. Total coliform significantly above the acceptable level of zero cfu was detected in 15% (9/60) of the bottled samples (p=0.004); and 70% (21/30) of sachet water (p=0.000). There was significantly higher prevalence of total coliform in sachet water compared to the bottled water (OR=13.2, 95% CI: 4.12-43.58). Also, more than half of the respondents, 56 % (237/423) preferred bottled to sachet water for drinking, because they perceived the latter as unsafe. In conclusion, about 15% of bottled water and 70% of sachet water samples in the retail outlets in Kampala city are likely to be contaminated with total coliform. Sachet water had significantly higher prevalence of total coliform compared to bottled water. Findings emphasize the need for repeated testing of packaged water at different processing levels at frequent intervals during the shelf life, community sensitization about recommended packaged water standards to improve their participation in quality surveillance and strengthening safety surveillance by Uganda National Bureau of Standards (UNBS).

641

FAECAL LACTOFERRIN, INFANT FOOD AND DIARRHEAL DISEASES IN A COHORT STUDY AMONG CHILDREN IN NORTHEASTERN BRAZIL

Aldo A. Lima[1], Alberto M. Soares[1], Noélia L. Lima[1], Leah J. Barrett[2], Richard L. Guerrant[2]

[1]Federal University of Ceara, Fortaleza, Brazil, [2]University of Virginia, Charlottesville, VA, United States

Lactoferrin is an iron-binding glycoprotein present as a major component of the secondary granules of polymorphonuclear neutrophils and also secreted by most mucosal membranes. Aim. The aim of this study was to determine the association of infant food and diarrheal diseases with faecal lactoferrin. A 45-month prospective cohort study, with infant food history and diarrheal diseases surveillance, was conducted in 184 children (0-3 years old) in an urban community in northeastern Brazil. Faecal lactoferrin (FL) was evaluated in 253 (23%) out of 1,091 specimens collected, using a latex agglutination test (LEUKO-TEST, TechLab, Blacksburg, VA). The test was considered positive for a title < or = 1:50 faecal dilution. Results. A total of 167 (66%) out of 253 samples was positive for FL. Children on exclusive breastfeeding (BF) had 100% (14/14; vs other food p=0.0003; Fisher's exact test), mixed BF 87% (78/90; vs other food p<0.0001; Chi-square test) and other food 52% (73/140), positive for FL. Children on BF plus mixed BF (any BF) had 88% (92/105; p<0.0001; Chi-square

test) FL positive compared to 51% (73/143) of children on other food. Children without any BF but with diarrheal diseases had 64% (58/90) FL positive compared to 30% (15/50; p<0.0001; Chi-square test) control children without any BF or diarrhea. Acute diarrhea episodes (AD; 2-6 days duration), prolonged diarrhea (Pro-AD; 7-13 days) and persistent diarrhea (PD; 14 days or more) had 63% (29/46; p=0.0012 vs control; Chi-square test), 65% (20/31; p=0.0023) and 69% (9/13; p=0.0095) FL positive, respectively. In conclusion, these data suggested an association of diarrheal diseases with intestinal inflammation. In addition, the results showed that any BF might influence on FL positive specimens, which can over estimate positive results. Further study of quantitative fecal lactoferrin concentrations may help distinguish low expected concentrations with breastmilk from higher expected concentrations with inflammatory diarrhea.

642

IN VITRO UNDERNUTRITION MODEL OF INTESTINAL EPITHELIAL CELL PROLIFERATION AND MIGRATION USING IEC-6 CELLS

Jefferson S. Oliveira[1], **Jones B. Neto**[1], Cirle A. Warren[2], James K. Roche[2], Aldo A. Lima[1], Richard L. Guerrant[3]

[1]Federal University of Ceara, Fortaleza, Brazil, [2]University of Virgina, Charlottesville, VA, United States, [3]University of Virginia, Charlottesville, VA, United States

Glutamine (Gln) is the preferred fuel source for enterocytes and is important in the maintenance of mucosal growth. Previous studies suggest that Gln modulates the response of the small intestine to systemic injury and infection. Enteroaggregative Escherichia coli (EAEC) has been implicated as a cause of persistent diarrhea among young children in developing countries. We hypothesize that the withdrawal of Gln and a decrease in fetal bovine serum (FBS), which contains amino acids important for cell viability including Gln, will cause the intestinal cells *in vitro* to be under severe stress that would be comparable to the animal model of malnutrition and would facilitate adherence of EAEC, thereby, increasing the severity of infection. To test this hypothesis we developed an *in vitro* model of "malnutrition". Rat intestinal epithelial cells (IEC-6) were cultured in media with or without Gln and supplemented with either 1% or 5% FBS. Cell proliferation and migration were measured after 6, 24, and 48 hours of exposure to the different media using WST1 colorimetric assay and cell counting software, respectively. IEC6 cells grown in 1% FBS or Gln-free media have significantly decreased proliferation at 6h, 24h, and 48h (p<0.05) compared with cells grown in media with 5% FBS and Gln 4mM (regular media). The optimal proliferation occurred at 24h (WST-1 absorbance was: 2.006 ± 0.071 SD, Gln(+) 5%FBS (positive control); 1.749 ± 0.087 SD, Gln(+) 1%FBS; 1.050 ± 0.085 SD, Gln(-) 5%FBS; 0.537 ± 0.055 SD, Gln(-) 1%FBS (negative control)). Furthermore, migration by cells in 1% FBS/Gln-free media was significantly less at 24h and 48h (p<0.05) (after 24h the mean of the count was: 2119.66 ± 384.07 SD; 1579.12 ± 117.142 SD; 1469.79 ± 55.664; 1220.78 ± 48.207, for the same groups respectively). These findings suggest our *in vitro* IEC-6 cell model may be applied in to the assessment of the impact of nutritional deprivation and glutamine intervention in *in vitro* models of bacterial adherence, such as EAEC infection.

643

EFFECTS OF ALANYL-GLUTAMINE IN IEC-6 CELLS CHALLENGED WITH *CLOSTRIDIUM DIFFICILE* TOXINS

Raphael S. Rodrigues[1], **Jones L. Neto**[1], Aldo A. Lima[1], Cirle A. Warren[2], Glynis Kolling[2], Richard L. Guerrant[2]

[1]Federal University of Ceara, Fortaleza, Brazil, [2]University of Virginia, Charlottesville, VA, United States

Clostridium difficile is the leading cause of antibiotic-associated diarrhea and pseudomembranous colitis, contributing to increased morbidity, mortality and cost because of prolonged hospitalization and illness. The bacteria produce toxins that causes necrosis and apoptosis in the intestinal epithelium through signaling pathways such as the Rho GTPase glycosylation and increases production of ERK. Glutamine is the most abundant amino acid in plasma and fuels the enterocyte to enable mucosal growth. Glutamine-induced cell signaling includes increases in antiapoptotic proteins (ERK, PKD) that inhibit cell death. The dipeptide alanylglutamine (AG) is more stable, 20-fold more soluble, and enters cells better than glutamine alone. Thus we examined the effects of AG when cells are exposed to the toxin A or B using IEC-6 cells. Migration and proliferation (WST-1) were assessed. Toxin A (10ng/ml) permits migration of cells but not in the same level of the control group. Toxin A at 1000ng/ml or 100ng/ml were largely lethal to these cells. Toxin B at 10ng/ml, 1ng/ml and 0,1ng/ml was cytotoxic in all groups.Toxin A (10ng/ml) inhibited both proliferation and migration by 20-40% and was chosen as the dose for further study. For Toxin B, 0,001ng/ml and 0,01ng/ml reduced migration and proliferation respectively by 20-40% and was used for further study. Toxins A or B reduced proliferation at 6 hours. AG given with toxin was protective on proliferation (p<0.05 in 18h and 24h) and migration (p<0.05 in 24h and 48h). AG given 24 hours before toxins had an earlier effect on proliferation (6h ; p<0.05). These studies suggest that AG supplementation may ameliorate *C. difficile* toxin-induced intestinal epithelial damage and may have a role in non-antimicrobial approaches to treat *C. difficile* infection such as the treatment with AG.

644

ANTIMICROBIAL SUSCEPTIBILITY, MECHANISMS OF RESISTANCE AND VIRULENCE FACTORS OF *SHIGELLA* STRAINS ISOLATED FROM PERUVIAN CHILDREN

Angela M. Lluque[1], Susan G. Mosquito[1], David Durand[1], Erik Mercado[1], Ana M. Prada[1], Carmen Contreras[1], Theresa J. Ochoa[1], Joaquim Ruiz[2]

[1]Instituto de Medicina Tropical Alexander Von Humboldt Universidad Peruana Cayetano Heredia, Lima, Peru, [2]CRESIB, Barcelona, Spain

Several reports showed that Shigella have been progressively acquiring resistence to commonly used and inexpensive antimicrobials. The aim of the study was to determine the antimicrobial susceptibility, mechanisms of resistance and virulence genes of Shigella strains isolated from Peruvian children under 5 years of age with and without diarrhea. 65 Shigella spp. isolates were assessed; 49 from diarrhea cases and 16 from healthy controls, from a cohort study in Lima, Peru. The isolates were serologically identified as 41 S. flexneri, 13 S. boydii, 8 S. sonnei and 3 S. dysenteriae. The susceptibility to five antimicrobial agents was tested by disk diffusion; the mechanisms of resistance and virulence genes were searched by PCR. A high proportion of Shigella isolates were resistant to cotrimoxazol (Sxt) (88%), tetracycline (Tet) (63%), ampicillin (Amp) (55%), and chloramphenicol (Chl) (45%). Only one isolate was resistant to nalidixic acid. Multi-drug resistance was present in 41% of S. flexneri, 8% S. boydii and in all S. sonnei isolates. Amp resistance was related to the presence of blaOXA(11/36), blaTEM (6/29) and blaCARB (9/36), no blaSHV were detected. Chl resistance was mainly related to cat (24/29), only one isolate presented cmlA and no floR were detected. Resistance to Tet was principally related to tetB (37/41) while tetA was founded in 2 isolates. SXT resistance was mostly related to sul2 (52/57), sul1 was only present in 3 isolates.

In the case of virulence genes, 97% (63/65) isolates present ipaH, the 2 isolates without this gene were S. boydii from the diarrhea group. The ipaBCD gene was detected in 56% of S. flexneri, 39% S. boydii, 13% S. sonnei and in the 3 isolates of S. dysenteriae. In conclusion, there is a high frequency of antimicrobial resistance to commonly used antibiotics, with multiple mechanisms of resistance. Quinolones remain as the drug of choice for the treatment of Shigella infections in Peru; however, development of resistance should be closely monitored.

645

COMPARISON OF SECRETED PROTEINS AND ACTIN POLYMERIZATION AMONG DIARRHEA AND CONTROL ENTEROPATHOGENIC *E. COLI* (EPEC) STRAINS ISOLATED FROM PERUVIAN CHILDREN

Carmen A. Contreras[1], Victor H. Bustamante[2], Theresa J. Ochoa[1], Maribel Riveros[1], Claudio F. Lanata[3], Thomas G. Cleary[4], Jose Luis Puente[2]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Universidad Nacional Autonoma de Mexico, Cuernavaca, Mexico, [3]Instituto de Investigacion Nutricional, Lima, Peru, [4]University of Texas School of Public Health, Houston, TX, United States

EPEC pathogenesis includes the production and translocation of bacterial proteins through a "needle complex" via a type III secretory system, and actin polymerization-associated intimate attachment and pedestal formation. The aim of this study was to compare the secretion of *E. coli* secreted proteins (EspA, EspB/D and EspC) and actin polymerization evaluated by FAS (Fluorescent actin stain) among diarrhea and control EPEC strains. 69 representative EPEC strains (46 diarrhea and 23 controls) isolated from children under 1 year of age living in Lima, Perú were analyzed for: Protein secretion (EspA, EspB/D and EspC) by SDS-PAGE and FAS test, evaluated in HEp-2 cells. The characterization of typical EPEC (tEPEC) and atypical EPEC (aEPEC) were determined by the presence of *bfp*A gene evaluated by PCR. EspB/D was the most frequently protein recovered in diarrhea (25/46, 54%) and control (17/23, 74%), as well as in tEPEC (10/13, 77%) and aEPEC (26/56, 46%). EspA was found with similar frequency in diarrhea and controls (22/46, 48% and 10/23, 43% respectively). EspA was more frequently found in tEPEC than in aEPEC (9/13, 69 vs 23/46, 50%). EspC was found with similar frequency among diarrhea cases and controls. The strains that secreted EspA and EspB/D proteins were more common in diarrhea cases than controls (19/46, 39% vs. 2/23, 9%, $p<0.05$). FAS was present in 16/46 (35%) of diarrhea vs. 4/23 (17%) of control samples, and in 9/13, 69% tEPEC vs. 11/56, 20% of aEPEC ($p<0.05$). In conclusion, our findings indicate that there is high heterogeneity among EPEC strains isolated from Peruvian children. Few EPEC strains secrete all 3 type III secretory proteins (EspA and EspB/D); however it correlates with diarrhea cases. The small frequency of actin polymerization among the isolated strains is due to the small frequency of tEPEC in our population.

646

ASSOCIATION OF TYPICAL ENTEROPATHOGENIC *ESCHERICHIA COLI* WITH TOTAL DIARRHEA EPISODES BUT NOT SUBTYPES OF EPISODES DURATION AMONG CHILDREN FROM FORTALEZA, CEARA, BRAZIL

Ila F. Lima[1], Josiane S. Quetz[1], Alexandre Havt[1], Eunice B. Carvalho[1], Alberto M. Soares[1], Noélia L. Lima[1], Rosa R. Mota[1], Richard L. Guerrant[2], Aldo A. Lima[1]

[1]Federal University of Ceara, Fortaleza, Brazil, [2]University of Virginia, Charlottesville, VA, United States

Enteropathogenic E. coli (EPEC) is a leading cause of infantile diarrhea in developing countries. Our recent data have shown and defined that prolonged acute diarrhea (Pro-AD; 7-13 days duration) and persistent diarrhea (PD; 14 or more days) episodes are risk factors for increased diarrhea burden in children. The aim of this work was to determine the prevalence of EPEC and its association with Pro-AD, PD or both types of episodes in children with (cases) and without diarrhea (controls) from urban areas in Fortaleza, Brazil. We analyzed stool samples collected from 249 children aging 2-36 months. Mothers provided information about the occurrence and duration of diarrhea episodes in the antecedent 14 days. Stool DNA was extracted by QIAamp DNA Stool Mini Kit (Qiagen, Valencia, CA). Diagnosis of EPEC was done by multiplex polymerase chain reaction (PCR, Qiagen) by the detection of eae (chromosomal, encodes intimin) and bfpA (located in eaf plasmid, encodes bundle-forming pili) genes. Approximately 30.1% (75/249) of studied children were positive for eae, 13.7% (34/249) for bfpA, and 35.3% (88/249) were positive for both genes. EPEC (eae+ and/or bfpA+) was significantly more detected in cases (89.2%, 74/83) than in controls (74.1%, 123/166) ($p=0.0076$). Typical EPEC (eae+ and bfpA+) was found in 65.1% (54/83) of cases and in 20.5% (34/166) of controls, and atypical EPEC (eae+ or bfpA+) was identified in 24.1% (20/83) of cases and in 53.6% (89/166) of controls ($p<0.0001$). There were no significant differences between EPEC pathotype neither between typical nor atypical EPEC regarding to duration of diarrhea episodes ($p<0.05$). In conclusion, typical EPEC strains were significantly associated with total diarrhea episodes in the studied population and no association was seen with EPEC diagnosis and the duration of diarrhea episodes. Therefore, EPEC strains seem not a risk factor for Pro-AD, PD or both.

647

CHOLERATOXIN AUGMENTS GENE EXPRESSIONS OF GLUTAMINE AND ALANYL-GLUTAMINE TRANSPORTERS IN RABBIT SMALL INTESTINE: THE ROLE OF GLUTAMINE RICH SMALL PEPTIDES-BASED ORAL REHYDRATION SOLUTIONS

Aldo A. Lima[1], Sandra S. Nunes-Monteiro[2], Alexandre Havt[1], Ila F. Lima[1], Eduardo E. Silva[1], Alberto M. Soares[1], Manuel C. Monteiro[1], Sean R. Moore[1], Richard L. Guerrant[3]

[1]Federal University of Ceara, Fortaleza, Brazil, [2]Federal University of Rio Grande do Norte, Natal, Brazil, [3]University of Virginia, Charlottesville, VA, United States

Glutamine (Gln) and alanyl-glutamine (AG) are potential candidates as nutritional supplement and replacing glucose in the oral rehydration and nutrition therapy for malnourish children. The aim of this study was to determine the effects of choleratoxin (CT) on genes transcriptions of glucose, glutamine and alanyl-glutamine transporters in rabbit small intestine and the role of glutamine rich small peptides-based oral rehydration solutions (ORS). Following RNeasy Mini Kit® protocol (Qiagen-Valencia, CA), total RNA was extracted from New Zealand rabbit small intestine. The relative genes transcriptions for the following intestinal transporters were determined with and without treatment with CT: PEPT1 (AG transporter); SGLT-1 (glucose transporter); SN-1 (glutamine transporter); and SN-2 (glutamine transporter). Intestinal electrolytes and water transports were evaluated in perfused intestinal loops and Ussing chamber (UC) experiments. The transcription of SGLT-1 was down-regulated, about 19 fold reduction, in tissue previously treated with CT. PEPT-1, SN-1 and SN-2 were up-regulated by 5, 213 and 124 fold increase in the same tissue treated with CT. UC results showed consistent improvement of sodium/hydrogen absorption induced by Gln, AG in CT treated tissues. Intestinal perfused experiments using Gln-, AG- or Gln rich peptides based ORS were also consistent with electrolytes and water (Gln = 183%; AG = 228%; Gln rich peptides = 165%) greater absorption in tissue treated with CT. In conclusion, these data suggested up-regulation of CT on genes transcription for Gln, AG and Gln rich small peptides in rabbit small intestine. The data also showed a consistent functional effect of electrolytes and water absorption using these substrates-based ORS in secretory diarrhea induced by choleratoxin.

648

A NEW MALNOURISHED APOE KO MURINE MODEL OF JM221 ENTEROAGGREGATIVE *ESCHERICHIA COLI* (EAEC) INFECTION

Jones B. Lima Neto[1], Jefferson S. Oliveira[1], Reinaldo Oriá[1], Aldo A. Lima[1], James P. Nataro[2], James Roche[3], Richard Guerrant[3]

[1]Federal University of Ceara, Fortaleza, Brazil, [2]University of Maryland, Baltimore, MD, United States, [3]Center for Global Health, University of Virginia, Charlottesville, VA, United States

Enteroaggregative Escherichia coli (EAEC) has been increasingly recognized as a cause of diarrhea in the developing and industrialized countries since its description in 1987, and the infection is characterized by watery, mucoid and secretory diarrhea. We compared Charles River C57Bl/6 wild type mice with ApoE knockout mice in order to assess the contribution of genetic lineage and nutritional status on the development of infection with the EAEC strain JM221. Mice were started on a low protein diet (2%) malnutrition protocol at day 21 of life. After 4 to 6 days of low protein diet, the malnourished (MN) mice were challenged with either the JM221 (virulent), media or HS (non-virulent) E. coli as negative controls. Bacteria were grown overnight (approximately 18 hours) in 1mL of Dulbecco's Modified Eagles Media (DMEM) and each mouse was challenged with 108 organisms in 100µL. Outcome measures were weight and shedding of the organism in the stools as determined by qPCR. Preliminary results show that MN ApoE knockout mice are more susceptible to EAEC strain JM221 (3 of 5, 60%, died) than to the negative control HS (1 of 4, 25%, died) in less than 48 hours after infection. In addition, JM221 also caused more diarrhea (2/5 vs 0/4 with HS) and tended to have impaired weight gain in the wild type animals in comparison with the HS challenged controls. Histology showed destruction of the epithelial architecture, as well as increased numbers of goblet cells in the colon in the JM221 infected animals that died. Malnourished wild type mice had transient weight loss with JM221, but no diarrhea or death. Quantitative PCR studies of fecal shedding and intensity of intestinal infection in this new model are underway.

649

SPOTTED FEVER GROUP RICKETTSIOSES IN MOROCCO

M'hammed Sarih[1], Najma Boudebouh[1], Touria Fatihi[2], Abdelfettah Chakib[2], Abdelfettah Chakib[2], Mohammed Hassar[1], Philippe Parola[3], Didier Raoult[3]

[1]Institut Pasteur du Maroc, Casablanca, Morocco, [2]Service des maladies infectieuses. CHU Ibno Rochd, Casablanca, Morocco, [3]WHO Collaborative Center for Rickettsial Reference and Research, Marseille, France

Rickettsioses are emerging infectious disease caused by rickettsiae in association with arthropods. There have been only few reports about epidemiology and clinical aspects of rickettsioses in this region. We report here a prospective study of clinical characteristics and course of 94 cas of rickettsioses diagnosed clinically in CHU Ibn rochd, Casablanca, Morocco, between May 2007 and December 2009. Sera and skin biopsies tested by reference methods including IF serology, Western immunoblotting combined with cross-adsorption, and molecular tools. A survey on the vector has been achieved in differents régions of Morocco. 679 specimens of various species of hard ticks colleted by direct removal from domestics animals (livestock, cattle, dogs) and by flannel flags dragged over vegetation have been analyzed by molecular methods on the presence of *Rickettsia sp.* The results show the presences of eight rickettsiae of the spotted fever group were identified, including 5 pathogens in ticks but that only the infection by *Rickettsia conorii* were detect among patients. These results increased our knowledge about the prevalence of Rikettsial pathogens in Morocco and provided information to understand the epidemiology of tick-borne diseases and may help to implement measures to control transmission to humans and animals in this region.

650

RAPID ASSESSMENT METHODS FOR TUNGIASIS AND PEDICULOSIS IN IMPOVERISHED COMMUNITIES

Liana Ariza

Federal University of Ceará, Rio de Janeiro, Brazil

Pediculosis and tungiasis are common in many resource-poor communities throughout the world. In these settings, despite the considerable burden caused by them, both neglected ectoparasitic diseases have been regarded by policy makers and health professionals as a problem of low priority. Consequently, prevalence and distribution of the disease are not documented in most endemic areas. To fill up this, gap rapid assessment methods were developed to estimate the prevalence and severity in endemic communities.

Recent studies from endemic areas in Brazil and Nigeria have shown that asking individuals about their pediculosis infestation status would give a highly accurate diagnosis. However, the accuracy varies greatly with geographic location and populations and is lower in developed market societies. For tungiasis, we have recently developed a rapid assessment method of high accuracy, based on identification of embedded Tunga penetrans fleas in the periungual sites of the feet. Considering the dynamic nature of the morphology of embedded jigger fleas, even lay people can diagnose the ectoparasitosis correctly. In areas where head lice infestations and tungiasis are endemic estimation of prevalence and severity of disease can be be based on simple and rapid approaches, and there is no need for resource-intensive and complex diagnostic procedures made by health professionals.

651

EFFECTS OF ROUTE OF INOCULATION OF *EHRLICHIA* ON LEVELS OF BACTEREMIA

Tais B. Saito, Lucas S. Blanton, Nagaraja Thirumalapura, Donald H. Bouyer, David H. Walker

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

Animal models have been developed for many emergent and re-emergent vector-borne diseases. For example, ehrlichial pathogenesis has been studied using the *Ehrlichia muris* or *Ixodes ovatus* ehrlichia (IOE)-C57BL/6 mouse model, but those models utilize intraperitoneal inoculation, which does not accurately reflect the natural route of transmission that occurs via tick feeding. The purpose of our study was to determine if the route of infection had an effect on the bacterial blood load in infected animals. In order to determine if the route of infection, intradermal (i.d.) versus intraperitoneal (i. p.), played a significant role, C57Bl/6 mice were inoculated with two *Ehrlichia* species, *E. muris* and IOE. The animals were infected via i.p. and i.d. inoculations using the same dose of bacteria, and blood samples were collected for analysis of bacteremia. For the *E. muris* infection, blood was collected on days 3, 6, 9, 12, 15, 20, and 30. Blood was collected from the IOE-infected mice on days 3, 5, 7, 9, and 12. The blood samples were processed for DNA purification and quantification by real time PCR using primers and probes specific to the *Ehrlichia dsb* gene. Our results indicated that after i. p. *E. muris* infection, ehrlichiae were first detected on day 3 p.i., and the highest level of bacteremia occurred on day 9, with decreased load on days 20 and 30 although persistent bacteremia was still detected. The *E. muris* i.d. infection model was less consistent, with the animals showing a wide variation of occurrence and concentration of bacteremia on days 12-15. IOE intradermal infection was similar to *E. muris*, showing an inconsistent infection pattern with bacteremia in only one animal in which IOE was detected on day 10, the same day as the animal became ill. In the IOE i. p. model of infection, the bacteria were detected on day 3 p. i. with a peak on day 5. The bacterial load decreased on day 7, 12 hours before they became ill. These results suggest that the route of infection can influence the bacteria load and course of infection.

652

SURVEY OF TICKS AND TICK-BORNE PATHOGENS IN NORTH DAKOTA

Nathan M. Russart, Jay A. Schroeder, **Jefferson A. Vaughan**

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

Outdoor enthusiasts in North Dakota acknowledge that ticks can be overwhelming during certain times of the year and within certain areas. However, there has been never been a systematic survey of ticks in North Dakota. Thus the abundance and distribution of different tick species within the state are ill-defined. Similarly, there are no data on the occurrence, distribution and prevalence of tick-borne pathogens in North Dakota ticks. With an increasing number of hunters and bird-watchers throughout the state in recent years, coupled with the possible range expansion of the deer tick, *Ixodes scapularis*, into the region, a statewide survey for ticks and tick-borne pathogens was initiated during the summer of 2010. Seven state wildlife management areas were selected for sampling, representing five different eco-regions; the Red River Valley, the Prairie Pothole region, the Turtle Mountains, the Missouri Coteau, and the Badlands. Ticks were collected via dragging (=questing tick population) and small mammal trapping (=feeding tick population). Ticks were identified to species, sex and lifestage. Ticks used for pathogen testing were washed and surface sterilized. DNA was extracted from pools of 3 to 10 con-specific ticks from each site. Multiple targets were used identify the presence of tick-borne microorganisms. All tick DNA was initially screened for the presence of bacterial symbionts using a PCR protocol designed to amplify a 800 bp fragment of the bacteria-wide 16S rRNA gene. *Dermacentor* DNA was screened for *Rickettsia* using a nested PCR designed to amplify a 434 bp fragment of the rickettsial 17 kDa gene. *Ixodes* DNA was screened with multiplex PCR for simultaneous detection of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* using primer sets designed to specifically the *B. burgdorferi* 23S rRNA and *A. phagocytophilum msp2* genes. As of this writing (May 2010), several questing adult *Ix. scapularis* ticks have been collected from the Red River Valley, confirming the presence of this tick species in eastern North Dakota.

653

RICKETTSIAL DISSEMINATION AND SPECIFIC TICK RESPONSE DURING TYPICAL AND ATYPICAL RICKETTSIAL INFECTION

Piyanate Sunyakumthorn

Louisiana State University, School of Veterinary Medicine, Baton Rouge, LA, United States

For tick-associated spotted fever group *Rickettsia*, ticks serve as vector and also reservoir hosts that can transmit *Rickettsia* via vertical and horizontal transmission; however, persistent transmission is dependent on specificity of the tick/*Rickettsia* relationship. Despite the apparent specificity between tick and *Rickettsia* species, the ability of different *Rickettsia* species to infect distinct tick genera has not been explored in detail. Therefore, the objective of this study is to monitor rickettsial dissemination in *Dermacentor variabilis* during *Rickettsia montanensis* (typical) and *R. amblyommii* (atypical) rickettsial infection. We hypothesize that only typical *Rickettsia* widely disseminates to tick tissues acquired for transmission and specific tick-derived molecules control rickettsial dissemination in ticks. We compare the dissemination of *R. montanensis* and *R. amblyommii* in female *D. variabilis* tissues (salivary gland, gut, and ovary) and characterize the tick tissue-specific response to different rickettsial species. Ticks are exposed to either *R. montanensis* or *R. amblyommii*. A *Rickettsia* species-specific qPCR and IFA are used to assess rickettsial dissemination. Additionally, transcription of selected tick molecules are compared among *Rickettsia*-uninfected and *Rickettsia*-infected tick tissues. The data indicate that tick molecules are differently regulated in a temporal and tissue specific manner. Gene expression profiles of β-thymosin, α-catenin, vATPase, Glutathione S-transferase 2, defensin-like protein, and Factor D-like serine proteinase genes are differently transcribed in ovarian tissues response to *R. amblyommii*, compared to *R. montanensis* infection. Studying the tissue-specific molecular interactions between ticks and rickettsiae will enhance our understanding of the key mechanisms that mediate transmission of *Rickettsia* by ticks, and the epidemiology of tick-borne rickettsial diseases.

654

EVALUATION OF METHYLATED RECOMBINANT OMPB FRAGMENTS AS REAGENTS FOR SERO-DIAGNOSIS OF *RICKETTSIA TYPHI* IN ELISA

Chien-Chung Chao, Zhiwen Zhang, Hua-Wei Chen, Elissa Mutumanje, Wei-Mei Ching

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

Rickettsia typhi, a Gram-negative, obligate, intracellular bacterium, is the causative agent of murine typhus. The bacteria possess a heavily methylated outer membrane protein B (OmpB), which has been shown to be an immunodominant antigen responsible for serological reactions and can elicit protective immune responses. We have previously expressed, purified and refolded two fragments (AN, aa 33-744 and K, aa 745-1353) that encompass the full length of OmpB. As the native OmpB have multiple methylation at various lysine residues, we also performed chemical methylation to artificially create methylated recombinant proteins (Me-AN and Me-K) to closely mimic native OmpB protein. Western blot analysis confirmed that Me-K exhibited increased sensitivity in comparison with K fragment without methylation. In this study, we evaluated the potential usage of these recombinant proteins for detecting anti-*R. typhi* antibody in patient sera either individually or in combination by ELISA. Among the samples we tested, some are only detected by AN fragment but not by K fragment and vice versa. The specificity of the assay was also evaluated by using negatives from the endemic area as well as patient sera confirmed of other diseases. The combination of both methylated AN and un-methylated K fragments showed higher sensitivity than any of the fragment used individually. In general, methylation of each fragment increased the sensitivity in comparison to un-methylated fragment, especially for IgM detection. However, in some cases, the methylation of K fragment did not exhibit better sensitivity than un-methylated K especially for IgG detection. Taken together, the results suggested that the combination of both methylated AN and un-methylated K provides the best sensitivity and specificity to detect antibody against *R. typhi* in patient sera.

655

INFECTION RATES OF COMMON TICKBORNE PATHOGENS IN LONE STAR TICKS (*AMBLYOMMA AMERICANUM*) AND AMERICAN DOG TICKS (*DERMACENTOR VARIABILIS*) FROM KENTUCKY

Charissa Fritzen[1], Junjun Huang[1], Kathleen Westby[1], James D. Freye[2], Brett Dunlap[2], John Dunn[1], Abelardo Moncayo[1]

[1]Tennessee Department of Health, Vector-Borne Diseases Section, Communicable and Environmental Diseases, Nashville, TN, United States, [2]United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, Madison, TN, United States

Ehrlichiosis, Rocky Mountain Spotted Fever (RMSF), and Lyme disease are tickborne diseases reported in Kentucky. Bacterial pathogens causing these diseases are *Ehrlichia chaffeensis, Rickettsia rickettsii,* and *Borrelia burgdorferi*, respectively. However, *Ehrlichia ewingii* has been documented as causing human ehrlichiosis in the United States. Additionally, other spotted fever group rickettsia, *R. amblyommii* and *R. parkeri*, have been associated with human rickettsiosis and could be a cause of human disease diagnosed as RMSF. Lastly, *Borrelia lonestari* has been associated with a Lyme-like illness referred to as southern tick associated rash illness, or STARI. *Amblyomma americanum* is the primary vector for *E. chaffeensis, E. ewingii, R. amblyommii* and *B. lonestari*. *Dermacentor variabilis* is the primary vector for *R. rickettsii* while *Ixodes scapularis* is the main vector

for *B. burgdorferi* in the eastern US. We conducted a survey to describe infection rates of tickborne pathogens in *A. americanum* and *D. variabilis* ticks collected in Kentucky. During 2007-2008, USDA-Wildlife Services collected 288 ticks, 179 *D. variabilis* and 109 *A. americanum*, from six counties in Kentucky. Ticks were removed from domestic and wild animals and were screened individually for *Borrelia spp.*, *E. chaffeensis*, and *E. ewingii* using conventional PCR. A real time PCR was used to screen the ticks for *Rickettsia spp*. followed by a conventional PCR. PCR positive ticks were subsequently tested by restricted fragment length polymorphism assay to determine rickettsial species. Forty-two (14.6%) ticks were PCR positive for a *Rickettsia spp*., 14 (4.86%) were positive for *E. chaffeensis,* and 4 (1.39%) were positive for *E. ewingii* infection. Two (0.69%) ticks, confirmed by sequence analysis, were positive for *Borrelia lonestari*. We described tick infection rates for common tickborne diseases in Kentucky. Physicians should be aware of the common tickborne diseases in their area of practice and include them in the differential diagnosis for patients with a febrile illness.

656

GENOTYPE IDENTIFICATION AND SEQUENCE ANALYSIS OF *ORIENTIA TSUTSUGAMUSHI* ISOLATED FROM SCRUB TYPHUS-INFECTED CHIGGER-MITE COLONIES

Ratree Takhampunya, Ampornpan Kengluecha, Taweesak Monkanna, Brian P. Evans, Jason H. Richardson

Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

Molecular characterization and pathogen-vector association studies were performed on twelve Orientia tsutsugamushi (Ot)-infected chigger colonies maintained in our laboratory in Thailand. Analyses were carried out on four Ot-infected Leptotrombidium chiangraiensis, 7 Ot-infected L. imphalum and 1 Ot-infected L. deliense colonies. The full-length 56-KDa type-specific antigen gene of O. tsutsugamushi was amplified and sequenced from 12 infected chigger lines followed by phylogenic analysis. The tree was constructed based on the full-length 56-KDa gene of 12 Ot sequences from infected chiggers along with reference sequences (Karp-type, Gilliam-type, TA716- and TA763-type strains) and scrub typhus sequences from a wide range of geographical regions available in GenBank databases. Moreover, the sequence diversity of O. tsutsugamushi and its associated vector species was also analyzed.

The clonal infection of O. tsutsugamushi was also analyzed from 2 Ot-infected chigger lines, L. chiangraiensis (Lc1) and L. imphalum (Li1). In so doing, we looked at the sequence diversity of the O. tsutsugamushi 56-KDa type-specific antigen gene from 2 infected chiggers and the infected mouse which developed symptoms after the bite of corresponding chiggers. Ten sequences of 56-KDa gene from each sample were selected and analyzed from cloning reactions. Our molecular characterization studies on laboratory colonies demonstrated that O. tsutsugamushi and its associated vector originated from naturally-infected chiggers collected from field rodents.

657

COLLAGENOLYTIC ACTIVITY RELATED TO METALLOPROTEASES (AND SERINE PROTEASES) IN *HYSTEROTHYLACIUM ADUNCUM* (NEMATODA: ANISAKIDAE), A WORLDWIDE FISH PARASITE

David Malagón[1], **F.J. Adroher**[1], Manuel Díaz-López[2], Rocío Benítez[1]

[1]Department of Parasitology, Faculty of Pharmacy, University of Granada, Granada, Spain, [2]Departamento de Biología Aplicada, Escuela Politécnica Superior, University of Almería, Almería, Spain

Proteases have a vital role in both the life cycle of parasites and the parasite-host relationship and are considered important virulence factors. In this study the presence of proteases with collagenolytic activity was investigated in the fish nematode *Hysterothylacium aduncum* during *in vitro* development. Collagenolytic activity was found in all studied developmental stages of the nematode (third- (L3), fourth- (L4) larval stages and adults). In L3, the activity was maximum at pH 6.5 and in the other stages, at 7.0. Pepsin is known to favour *in vitro* development of the worm, but, in this study, collagenolytic activity was shown to be significantly greater when no pepsin was added to the culture medium (at pH 6.5, $p = 0.011$). At pH 7.0 most activity was observed in the immature adult, after the final moult, suggesting that the collagenolytic activity may be involved in the remodelling of the cuticle and in sexual maturity. On the other hand, at pH 6.5, activity may be related to tissue migration by L3 within the host. Using specific inhibitors, it was demonstrated that most of the collagenolytic activity detected in all the developmental stages was due to metalloproteases (40-100%), although serine proteases were also detected in L4 and adults (10-30%).

658

BRONCHIAL WALL REMODELING IS REDUCED BY DEXAMETHASONE TREATMENT DURING LARVAE PULMONARY MIGRATION OF *STRONGYLOIDES VENEZUELENSIS* IN RATS

Cristiane Tefe-Silva, Cristina T. Beneli, Eleuza R. Machado, Marlene T. Ueta, Elaine M. Floriano, Carlos A. Sorgi, Lúcia H. Faccioli, Simone G. Ramos

University of Sao Paulo, Ribeirao Preto, Brazil

Strongyloidiasis is an intestinal parasitosis with an obligatory pulmonary cycle. The immune response is associated with Th2-type activated CD4 T cells, which amplify the cellular response through the secretion of cytokines in an attempt to eliminate the parasite. Although the inflammatory response seems similar to asthma with eosinophilia, elevated serum IgE and airway inflammation, the possible mechanisms of bronchial remodeling during pulmonary migration of larvae has not been established. The aim of this study was to delineate the principal mechanisms involved in bronchial wall remodeling occurring during the passage of *Strongyloides venezuelensis* larvae in rat lungs and to determine the ability of dexamethasone treatment to interfere with this process. Animals were divided into four groups: a control group, (C); a control plus dexamethasone group (CD); an infected group (I); and an infected plus dexamethasone group (ID). The (I) and (ID) groups were inoculated with 9,000 *S. venezuelensis* larvae. The (CD) and (ID) groups received 2 mg/kg of dexamethasone. At 1, 3, 5, 7, 14 and 21 days, the animals were killed. Morphologic and morphometric analyses with routine stains and immunohistochemistry were conducted, and cytokines were evaluated by ELISA. Goblet cell and smooth muscle hyperplasia, collagen deposition and inflammatory infiltrate (primarily composed of eosinophils and mast cells) were seen in thickenings of the bronchial wall, and IL-1β, IL-4 and VEGF levels were elevated throughout the course of infection. Both the morphologic alterations and the immunomodulatory response to the infection were drastically reduced in the dexamethasone-treated animals. In conclusion, our results strongly indicate that airway remodeling occurs during passage of *S. venezuelensis* into the lungs as a consequence of T helper type 2 inflammation. Furthermore, dexamethasone treatment can inhibit this process, acting primarily by suppressing cytokines.

659

PRELIMINARY CHARACTERIZATION OF TWO PROTEIN TARGETS WITH POTENTIAL USE FOR THE DIAGNOSIS OF ANGIOSTRONGYLIASIS

Alessandra L. Morassutti[1], Paulo M. Pinto[1], Alexandre J. da Silva[2], Patricia Wilkins[2], Carlos Graeff-Teixeira[1]

[1]Faculdade de Biociências da PUCRS, Porto Alegre, Brazil, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States

The nematode species *Angiostrongylus cantonensis* and *A. costaricensis* are agents of human eosinophilic meningitis and

abdominal angiostrongyliasis, respectively. Both infections have been reported worldwide, especially eosinophilic meningitis, which has also been reported in the United States. Parasitological diagnosis of angiostrongyliasis is rarely possible, since larvae are retained in human tissues due to a severe inflammatory reaction. Instead, both serological and real-time PCR tests have been employed for diagnosis. To improve serological diagnosis for angiostrongyliasis, excretory-secretory (ES) antigens were purified and identified. The ES was prepared by culturing worms in RPMI medium with antibiotics at 37ºC in 5% CO2; fresh medium was replaced every 24 hours. The supernatant resulting from centrifugation at 15000 x g for 10min was precipitated with TCA and the proteins were solubilized into SDS-PAGE sample buffer. Samples were in-gel rehydrated and second dimension separation was performed in SDS-PAGE gels (12.5% acrylamide). The proteins were stained or transferred onto nitrocellulose membrane, incubated for 1 hr in 5% skim-milk at room temperature and then incubated for 2h with a 1:200 pooled serum samples from patients with a confirmed histological diagnosis of angiostrongyliasis. The spots that reacted with the pooled sera were manually excised from 2-D gels, digested with trypsin and applied to a liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis using the ESI-Q-TOF equipment. The peptides obtained from two different spots matched with MTM-3 protein of *Caenorhabditis briggsae* and LEC-5 protein of *C. elegans*. Further studies to verify the specificity of these targets for diagnosis of angiostrongyliasis are under way.

660

TAX/*HBZ* MRNA RATIO AND REGULATORY T-CELL EXPANSION *IN VIVO* ARE HIGHLY ASSOCIATED IN HTLV-1/ *STRONGYLOIDES* CO-INFECTION

Giovanni Lopez[1], Carlos Rodríguez[1], Nicolás Barrós[1], César Sánchez[1], Martín Montes[1], Elsa González[1], Daniel Clark[2], Kristien Verdonck[1], Eduardo Gotuzzo[1]

[1]Instituto de Medicina Tropical "Alexander von Humboldt", Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Laboratorios de Investigación y Desarrollo (LID), Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru

Strongyloides stercoralis infection might promote the clonal expansion of HTLV-1 infected cells. HTLV-1 in turn leads to an increase of regulatory T cells (Treg), which down regulates the immune response to the parasites. We have shown that the proportion of Treg is higher in patients with strongyloidiasis co-infected with HTLV-1 than in those with only *Strongyloides stercoralis* (PLos. Neglected Tropical Diseases. 2009; 3(6): e456). We hypothesized that the expression patterns of TAX and HBZ, two key proteins important for viral activity, determine the clonal expansion of Treg. We compared viral *tax* and *hbz* mRNA levels and the proportion of Treg in two groups of patients: (1) HTLV-1 asymptomatic carriers with no history of Strongyloidiasis [AC] (n=4); and (2) patients with a history of HTLV-1/ *Strongyloides* co-infection [SS] (n=8). Peripheral blood mononuclear cells were isolated to measure HTLV-1 proviral load, *tax* and *hbz* mRNA levels by qPCR, and the proportion of Treg by flow cytometry. Statistical analysis was based on non-parametric tests. Between both groups, there were differences in the proportion of infected Treg [AC: 6.31, SS: 10.44, p =0.035]. The proportion of Treg positively correlated with the HTLV-1 proviral load [Spearman r =0.687, p=0.01] and higher with the *tax/hbz* mRNA ratio [Spearman r=0.780, p=0.002], whereas it correlated negatively with the *hbz* mRNA [Spearman r = -0.615, p=0.013]. We concluded that the two groups of patients studied have a distinctive pattern for Treg; the correlations of HTLV-1 proviral load, *tax/hbz* mRNA ratio with the proportion of Treg suggest that provirus *tax* mRNA and *Hbz* mRNA expression during co-infection *in vivo* is associated with an extensive proliferation of infected clones with Treg phenotype, might be induced when *tax* expression predominates over *Hbz* expression in a context of high viral activity.

661

ANGIOSTRONGYLUS CANTONENSIS AND DETECT IN SNAIL INTERMEDIATE HOST

Angiostrongyliasis, caused by infection with *Angiostrongylus cantonensis*, is a potentially fatal food-borne disease. Outbreaks have become increasing common in China due to the spread of efficient intermediate host snails. *A. cantonensis* was discovered in the pulmonary arteries and hearts of domestic rats in Guangzhou (Canton), China, by Chen in 1933. Human acquire infections by ingestion of raw or undercooked snails or slugs, paratenic hosts such as prawns, or contaminated vegetables that contaminated with the infectious third-stage larvae of the worm. During 1997 to 2008, nine outbreaks of the disease have been reported in the mainland of China and three in Taiwan. The biggest outbreak in the capital Beijing in 2006 demonstrated that angiostrongyliasis had moved beyond its traditional endemic areas located in the southeastern coastal regions of China. *P. canaliculata*, which has high compatibility of *A. cantonensis*, is believed to be one of the closely associated snail intermediate hosts with angiostrongyliasis in China.Three methods, e.g. tissue homogenate, enzyme digestion and lung-microscopy are major detection methods in detecting larva of *A. cantonensis* from the snails. But the first two methods are time-consuming, due to their difficulty to digest the muscle of the mollusks by enzymes. Though the third method is fast, all those methods required skilled staff and are lack of specificity in large scale detection. Newly developed PCR-based assays are able to overcome abovementioned shortcomings with its greater sensitivity, especially in the setting of low burden of parasite infection. We have established a multiplex PCR assay to detect the infection and provided an alternative method with a higher sensitive rate in detection of *A. cantonensis* in *P. canaliculata*. Despite those advantages of developed techniques, the high cost of reagents, equipment, and quality assurance hindered the application of PCR-based assay in detection of *A. cantonensis* infection. Additionally, a small sampling around 100 mg from an individual snail whose weight can reach to 70 g, can not accurate tell positive or negative. Individual detection by multiplex PCR would be a heavy workload and high cost. Pooling field specimens could reduce the number of assay and thus increase the efficiency in detecting and screening pathogen infections by polymerase chain reaction (PCR)-based assay. We investigated a pooling strategy on diagnosis of *A. cantonensis* in *P. canaliculata*. Two settings of specimens were prepared, divided into portions and detected by multiplex PCR. Specimens A was 0.4490 g positive lung tissue of 28 larval nodes from 4 snails mixed with 1.310 g negative lung tissue from 6 snails and divided into 32 portions. Specimens B was 0.5448 g positive lung tissue with 26 larval nodes from 2 snails mixed with 1.092 g negative from 7 snails and divided into 48 portions. Samples were detected by multiplex PCR. Repeated sampling was performed and sample size-accumulated positive rate curves were drawn. According to the sample size-accumulated positive rate curves, the appropriate sample size of the two specimens were 18 and 15, respectively, which is 0.36~0.58 to the total sample size. These test characteristics and the relevant factors to the sample size would need to be determined in much larger studies and more appropriately in field populations. The result indicates the number of larval node is not the most and only factor to the sample size. And it implies the feasibility to detect *A. cantonensis* in *P. canaliculata* by pooling strategy. In conclusion, by using the pooling strategy to detect the infection of *A. cantonensis* in *P. canaliculata,* we are not only able to carry out detection work in large sample, but also reduce the amount of detection samples.

662

GEOHELMINTHS AND HIV AMONG PREGNANT WOMEN FROM COASTAL KENYA: THE ASSOCIATION WITH MATERNAL HEALTH

Ernest Midega

Case Western Reserve University, Mombasa, Kenya

Both HIV and geohelminth infections are associated with maternal morbidity and impaired fetal development. Geohelminth infections in pregnancy have been associated with maternal anemia and impaired nutritional status. An overlapping distribution of the two pathogens becomes important if there is a synergistic effect of concomitant infections.Our study is aimed at describing geohelminth incidence among expectant mothers in a Sub-Saharan site, coastal Kenya to identify factors associated with variability in distribution, as well as to describe the association of cross-infection with maternal morbidity. This information will provide an evidence base to guide antenatal health care policy, particularly as it relates to the management of co-infections in HIV in expectant mothers. 1000 expectant women were recruited in their second trimester through two district hospitals in coastal Kenya, representing one urban and one rural site. Data collected at antenatal clinic includes HIV status, socio-economic status, blood and stool samples. The role of co-infection will be investigated through its association with maternal morbidity (anemia and nutritional status). Preliminary analysis has described a number of features of infection that suggest need for targeted guidelines for screening and treatment of geohelminth infection in pregnancy. The rural sample was characterized by a higher and more varied worm burden (risk of being infected rural R.R=3.3 vs. urban R.R=0.109). The association between HIV status and worm infection was not in the expected direction with P =2.1. This will be discussed. Both HIV and worm infection was related to the Hb levels of the mothers, as well as their nutritional status. The risk of low Hb or weight being associated with co-infection was greater single infections with R.R of =1.2.

663

MORPHOLOGICAL CHANGES OF *ASCARIS* SPP. OVA DURING THEIR DEVELOPMENT OUT OF THE HUMAN HOST

Ligia M. Cruz, Michael Allanson, Ricardo Izurieta

University of South Florida, Tampa, FL, United States

Ascaris lumbricoides is of particular importance to public health as it causes a great burden of disease in children in developing countries. Information on the infective stage and the pathological damage caused by the parasite is abundant and widely available in the literature; while information about early embryonic development and its life cycle out of the human host is limited. The objective of this study was to register and describe the morphological changes within the parasite ova during incubation *in vitro* at 28 C°.*A. suum* were used as a model for *A. lumbricoides.* Five milliliters of 0.1N H_2SO_4 was prepared in a 50ml centrifuge tube (4000 ova/ml) and placed in an incubator at 28 C° in the dark, for 21 days. Every day, subsamples of approximately 100 *Ascaris suum* ova were taken from the incubation solution for microscopic evaluation. Development, morphological changes and viability of the first 40 ova observed were registered in a log sheet and documented with photos. Twelve stages of development were identified within the ova: 1-Cell, 2-Cell, 3-Cell, 4-Cell, Early Morula, Late Morula, Blastula, Gastrula, Pre-larvae 1, Pre-larvae 2, Larvae 1, and Larvae 2. Each stage was observed for at least three continuous days. By the end of the first week most ova observed were in Late Morula stage (72.5%); on day 14 of incubation, 90% had developed to Larvae-1 stage and by day 16, 62.5% had developed to Larvae-2 stage. No difference was found between viability recorded from day 5 to 20 of incubation, and viability reported after three weeks of incubation (Z test for proportions, 99% CI). In conclusion, ***A. suum*** ova went through clearly identified morphological changes at different speed of development. At the end of incubation, 21 days, 100% of ova observed were in Larvae 2 stage. Two new additional stages of development were identified: Prelarvae-1(larvae coiled creating no more than one concentric ring) and Prelarvae-2 (larvae coiled creating at least one and a half concentric ring). Viability of ***Ascaris*** spp. ova may be established at earlier stages of incubation.

664

AGRICULTURAL PRACTICES ARE RELATED TO HUMAN HELMINTH INFECTION IN SUB-SAHARAN AFRICA

Naomi Hauser[1], Innocent B. Rwego[1], Derek Meyer[2], Elizabeth Canfield[3], Tony L. Goldberg[4], J. Byaruhanga[5], A. Nyamwija[5], Thomas R. Gillespie[1]

[1]Rollins School of Public Health, Emory University, Atlanta, GA, United States, [2]University of Illinois College of Medicine, Chicago, IL, United States, [3]Department of Environmental Studies, Emory University, Atlanta, GA, United States, [4]University of Wisconsin School of Veterinary Medicine, Madison, WI, United States, [5]Makere University's Biological Field Station, Kibale National Park, Uganda

Sub-Saharan Africa is a region of high species diversity and frequent gastrointestinal (GI) helminth infection. To better understand the relationship between behavior and infection, occupational and farming practices were examined relative to parasitic nematode infection in Kabarole District in western Uganda, a region typical of developing rural economies characterized by very close human-livestock associations, making it ideal for a study of zoonoses. One hundred eighty-four individuals from nine villages were included in the analysis. Subjects were surveyed and screened for GI helminthes via fecal flotation and sedimentation methods. Logistic regression and correlation analyses were used to determine associations between villages, agricultural practices, and helminth infection. Infection with any parasite was found to be associated with tending cattle (p=0.093) and pigs (p=0.089); *Trichuris* sp. was associated with doing fieldwork less than daily (p=0.0068), tending goats (p=0.055), and being an agricultural worker (p=0.015) or student (p= 0.086); *Oesophagostomum* sp. was associated with tending pigs (p=0.078); and *Trichostrongylus* sp. was associated with tending pigs ever (p=0.028), tending pigs (p=0.0035) and cattle (p=0.096) daily, and being an agricultural worker (p=0.091). The village of Kamakune I showed the highest prevalence of infection overall (68%) and Kamakune II showed the lowest (14%). Among the thirty people from Nyaruzigati, significant positive associations were found between the number of pigs at a household and *Ascaris* sp. (p=0.0028) and *Trichuris* sp. (p=0.033). Results of this study suggest that, even in small numbers, pigs are highly associated with human infection with multiple GI helminths. Other farming practices are similarly associated with helminth infections to lesser degrees. These results could prove important for restructuring the agricultural framework in rural sub-Saharan Africa to significantly decrease the human risk of GI helminth infection and associated morbidity and mortality.

665

CHILDREN PREVALENCE AND RISK FACTOR OF SOIL TRANSMITTED INFECTION IN KINSHASA (DRCONGO)

Solange E. Umesumbu, Thierry L. Bobanga, Alain N. Mandoko, Mimie M. Zanga, Ange L. Landela, E. Metelo, O. Fataki, Celestin N. Nsibu

Universite de Kinshasa, Kinshasa, The Democratic Republic of the Congo

Soil Transmitted Helminths (STH) infestation is prevalent in underdeveloped countries and may affect children's growth. The overall burden of disease is estimated to Ancylostoma sp *A.lumbricoides* and *T. trichiura* and 39 million DALYS. The objective of this study was to determine the prevalence and risk factors of intestinal STH in children of preschool and school age. We conducted a cross sectional study with a standardized sampling proportion in Kinshasa. It was divided into 5 strata with selected households were visited, and which stool samples were obtained for qualitative STH analysis. Questionnaire data on various demographic,

housing and lifestyle variables were available. The Pearson chi-square was used for comparison of frequencies at a significance level of 5%. Logistic regression identified different risk factors. 1160 stool samples were collected and examined. The prevalence of any STH infection was 17.4% and 38.5% respectively for preschool age children (0-5years) and those of school age (6-15years) with a very highly significant difference (p <0, 0001), with an average of 28.8%. *Ascaris lumbricoides* and *Trichuris trichiura* were the common STH with respectively 22,4% and 13,7% the common STH with. Age below 5 years (OR = 0.32, CI = 0.24-0.42, P <0.000), Community-Directed Treatment with Ivermectin area (CDTI) (OR = 0.54, CI = 0.38-0.76, p <0.000) and washing hands after defecation (OR = 0.73, CI = 0.54 to 0.98, P <0.039) were associated with reduced risk of STH infection; the low level of maternal education (OR = 1.45, CI = 1.10 -1.92, P <0.008) and not washing food before consumption (OR = 1, CI = 1.09-1.95,P <0.011) were associated with increased risk of STH infection. Approximately one fifth preschoolers and half of those of school age have been infected by different species of STH. We found a reduced risk of STH infection in relation with hygiene practices and safe supply of water. The national de-worming approach must be changed including of school age.

666

BILE SALT STIMULATED LIPASE GENOTYPES IN GHANAIAN COUPLES DISCORDANT FOR HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 INFECTION

Yvonne Affram[1], Martijn Martijn[2], Georgios Pollakis[2], Kwamena W. Sagoe[1], Julius A. Mingle[1], William K. Ampofo[3], William A. Paxton[2], Barnor Jacob[3]

[1]University Of Ghana Medical School, College of Health Sciences, University of Ghana, Accra, Ghana, [2]Experimental Virology Unit, Academic Medical Centre (Amc), University of Amsterdam., Amsterdam, The Netherlands, [3]Virology Department; Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Accra, Ghana

Some individuals remain HIV-1 seronegative despite multiple sexual exposures to HIV-1 virus. This study analyzed the possible role of bile salt stimulated lipase (BSSL) genotypes in the lack of HIV-1 transmission in Ghanaian HIV-1 serologically discordant couples (SDCs). BSSL is a Lewis X-carrying glycoprotein secreted by the pancreas and present in human milk, the testes, adrenals and blood plasma of humans. BSSL has been postulated to have variant capacity to bind Dendritic cell-specific ICAM-3 grabbing non-integrin (DC-SIGN) and potentially block viral transmission across a mucosal surface. A total of 32 couples were enrolled in the study. These comprised of 12 SDCs and 20 serologically concordant couples (SCC). Five milliliters of blood was taken from couples. HIV-1 antibody testing was done using Abbott HIV-1/2 Determine assay and confirmed with Innolia HIV-1/2 assay. HIV-1 negative serostatus of discordant negative partners was confirmed by polymerase chain reaction (PCR) and BSSL genotypes of all couples were also identified by PCR. HIV antibody testing with PCR confirmation revealed 8 SDC and 24 SCC. BSSL genotypes were grouped into high high (HH), high low (HL) and low low (LL) genotypes based on the number of repeats (ranged from 6 to 19 repeats; 16 repeats or more was denoted as high (H) and less than 16 repeats was low (L)). Each patient had 2 types of the repeats. Fifty five percent of SDCs had HL genotype found to be associated with strong binding of BSSL to DC-SIGN, 20% had HH genotype and 25% had LL genotype both of which are associated with weak binding of BSSL to DC-SIGN. On the other hand, 40% of SCCs had HL genotype, 45% had LL genotype and 15% had HH genotype. In conclusion, SDCs could be more protected against HIV-1 transmission from DC-SIGN to CD_4 cells than SCCs.

667

DRUG RESISTANCE MONITORING IN HIV-1 INFECTED PATIENTS ON ART'S AT KOFORIDUA IN GHANA

Jacob Barnor[1], Jim Brandful[1], William Ampofo[1], Alexander Nyarko[1], **Michael Alale**[1], Evelyn Bonney[1], Kofi Bonney[1], Samson Badu Ofori[2], Nobuo Ohta[3], Koichi Ishikawa[3], Shoji Yamaoka3[3]

[1]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [2]HIV/AIDS Clinic, Government Regional Hospital, Koforidua, Koforidua, Ghana, [3]Molecular Virology Department, Tokyo Medical and Dental University, Tokyo, Japan

Since the first case of HIV was reported in Ghana in 1986, the number of cases and prevalence of the disease has increased at astronomical rates. Pragmatic interventions led to a gradual decline in the prevalence rates (currently, 1.8%) in the last couple of years. These interventions, including antiretroviral therapy (ART), were directed towards reducing new infections and improving the quality of life of infected persons. The objective of this study was to identify mutations related to drug resistance and to monitor plasma HIV-1 viral load as a marker of treatment success. Patients on ART were recruited with their consent. Blood samples drawn from the patients were analyzed for CD4/CD8 counts. Viral RNA was extracted from the plasma for viral load quantification and nucleotide sequencing done for drug related mutations. Majority of patients were within 21-50 years old with an average age of 38 and 42 in females and males respectively. 70.2 %(n=424) of patients were females. The prevailing strain of HIV is the CRFO_2AG. ART suppressed HIV in 86 %(177) of patients while the remaining 14 %(29) showed high viral loads ranging between 10^3-10^6, 6 of which were confirmed as having drug resistant mutations by the Stanford software. In conclusion, currently, ARTs designed specifically against HIV-1 B strains had shown to be effective against the CRFO_2AG strain in Ghana. However, the emergence of drug resistant mutants could create a paradigm shift in this success story if not controlled.

668

LOW APOPTOSIS AND G2 PHASE ARREST-INDUCING EFFECTS OF VPR MUTATED FRAGMENTS ON JURKAT CELLS

Diallo Mamadou[1], Zheng Yu-huang[2], He Yan[2], Zhou Hua-ying[2], Liu Meng[1], Chen Xia[1], Chen Zi[1]

[1]Department of Infectious Diseases, Laboratory of AIDS Research, Xiangya Second Hospital, Central South University, Changsha, China, [2]Xiangya Second Hospital, Central South University, Changsha, China

Vpr (viral protein R) is a vital HIV-1 accessory protein with multiple functions in the viral life cycle, the pathogenesis and in the induction of apoptosis and cell G2 arrest. Recent studies have shown that mutation of certain amino acid sequences in the C-terminal domain might influence the course of disease progression and attenuate its apoptosis-inducing capacity on infected cells. The present study was designed to transfect Jurkat cells with several HIV-1 vpr fragments carrying specific mutation sites, and to observe their ability of inducing distinctively apoptosis and G2 arrest. 14 vpr variant fragments were chosen from Chinese HIV-1 infected individuals. After PCR amplification, the products were purified and double digested with Hind III and BamHI. The pcDNA3.1 (+) eukaryotic expression plasmid was used for the ligation and transduction experiments. The recombinant plasmids were transiently transfected into Jurkat cells with liposomes; blank cells and empty vector cells established as control. mRNA expression of target genes was detected by RT-PCR, the DNA content, the percentages of apoptosis and the cell G2 arrest monitored by flow cytometry. Cells transfected with vpr fragments presenting 70V, 85P, 86G or 94G mutations displayed reduced percentages of apoptosis and G2 arrest when compared to the wild consensus genes. In conclusion, we found that although the HIV vpr could induce apoptosis and G2 arrest, but certain mutations such like 70V, 85P, 86G or 94G could drastically reduce this ability hence rising up a great interest for further research on gene therapy.

669

THE INCIDENCE OF OPPORTUNISTIC AND OTHER INFECTIONS IN HIV-1 INFECTED CAMBODIAN CHILDREN IN CORRELATION WITH CD4 CELL PERCENTAGE

Julia Vujcikova[1], **Vladimir Krcmery**[1], Andrea Shahum[1], Peter Kisac[2], Juraj Benca[1], Irad Beldjebel[1], Jaroslava Sokolova[2], Silvia Seckova[1], Veronika Sladeckova[1]

[1]St. Maximilian Kolbe HIV Tropical Programme Phnom Penh, Cambodia and St. Elizabeth University College, Bratislava, Slovakia, [2]St. Elizabeth University College, Bratislava, Slovakia

Infectious complications of HIV infection are important indicators of disease progression. In pre- HAART era opportunistic infections (OIs) and other infections were the critical cause of morbidity and potential mortality of children with HIV infection. In HAART era, OIs continue to be the presenting symptom of HIV infection among children whose HIV- exposure status is not known, in whom drug resistance causes clinical failure of treatment, because of poor adherence or suboptimal care. Multiple drug interactions may decrease treatment efficacy also. In our study we report the occurrence of OIs at the St. Max Kolbe Clinic in Phnom Penh, Cambodia, within 5 years. Between October 2003 and October 2008, 91 HIV- infected Cambodian children were enrolled into retrospective study. Our aim was to estimate the incidence of 12 targeted OIs and other infections occurring with association with CD4 cell percentages in a country with limited resources. In case of 66 children on first line of HAART we compared the occurrence of OIs and other infections and the duration of HAART use. For each of events, we calculated the incidence rate, 95% confidence interval per 100 person-years. The median of age of children at the presentation was 6.75 years. Average CD4 cell percentage at the presentation was 10.6% (median 8%, IQR 1.67-16.85), 67% of children had severe immunosuppression (CD4 < 15%). Severe form of malnutrition was diagnosed in case of 23% of children. The most common first time infections in the group with CD4 < 15% were pneumonia, pulmonary TB, fungal skin infections (dermatophyte infections), oropharyngeal candidiasis. The frequency of OIs and other infections generally showed statistically significant decreasing trends with increasing CD4%. The incidence of pneumonia, otitis media and dermatophyte infections remained still high in contrast to candidiasis, which correlated with low CD4%. Similarly correlated the occurrence of herpes zoster, but still kept to be present in the group with CD4>25%. We did not observe infections as PCP, DMAC, CMV, toxoplasmosis or cryptococcosis. The high incidence of OIs and other infections in the group of children with low CD4% is comparable with other studies. Our diagnostic facilities were limited. We found HAART effective for HIV infected children in resource- limited setting despite the initiation of the treatment in the advanced stage of the disease.

670

NEVIRAPINE-USE AND EVALUATION OF AN HIV PMTCT PROGRAM AT KILIMANJARO CHRISTAIN MEDICAL CENTRE (KCMC)

Anastasia Grivoyannis[1], Josephine Maleo[2], Olola Oneko[2]

[1]Weill Cornell Medical College, New York, NY, United States, [2]Kilimanjaro Christian Medical Centre, Moshi, United Republic of Tanzania

Implementation of a Prevention of Mother to Child Transmission (PMTCT) program: designed to identify and treat pregnant women at high risk for transmitting HIV to their offspring by vertical transmission was evaluated in the Obstetrics & Gynecology Department at Kilimanjaro Christian Medical Centre (KCMC) in Moshi, Tanzania from 2006-2007. The study population was drawn from women giving birth at KCMC which serves ~17,000 people (of this 9,600 are women of childbearing age). Data from 3,170 women admitted to the Labor Ward from July 2006 to July 2007 were abstracted including: maternal HIV status at time of delivery, extent of mother's use of ARVs throughout pregnancy, method of delivery, infant feeding choice (breast milk vs. formula), estimated blood loss, maternal age & parity, and newborn statistics (i.e. gestational age, birthweight, APGAR score). Of the 3,170 deliveries, 174 (5.5%) women were HIV+ at the time of delivery, 2,435 (76.8%) were HIV negative. Of the HIV+ women, 133 (76%) received nevirapine before delivery, 3 were undocumented, 1 refused therapy, and 37 did not receive nevirapine because they were admitted after the second stage of labor, during which time nevirapine administration is not indicated. If the mother's HIV status was unknown, as was the case for the remaining 561 (17.7%) women, precautions for prevention of HIV transmission during delivery were not followed. Of the 561 women, 307 were subsequently tested and 29 of these were found to be HIV+. Guidelines were successful in screening 82% of the population at risk but failed to help those 37 women who did not arrive to Labor & Delivery in time, and 29 who tested HIV+ after delivery. Also, there are 254 women, for whom HIV status remains unknown. In order to ensure the success of PMTCT program implementation, the department must identify methods to expand HIV testing so that high risk deliveries can be better identified and treated obeying PMTCT protocol.

671

THE INTERDICTION PROJECT: AN INNOVATIVE PROGRAM FOR HIV+ PERSONS PRESENTING WITH A NEW STD

Samuel Frimpong, M. Maximillion Wilson

Duval County Health Department, Jacksonville, FL, United States

STD/HIV field investigations reveal that some persons with HIV infection have unprotected sex, multiple anonymous sex partners, and don't disclose their HIV positive status. Recurrent STDs are common among these persons, making HIV transmission more likely. The Interdiction Project is a clinic-based, individual-level HIV intervention that combines linkage for treatment adherence/risk reduction education and testing with ongoing monitoring of patient care and epidemiological data systems. This project targets HIV positive persons who present with a new STD. This presentation describes processes for establishing a program to reduce the spread of HIV and repeat STD infections among HIV positive persons. The program integrates use of existing data systems and personnel (medical providers, STD field staff, and HIV health educators). Strategies for overcoming client identification/retention and institutional barriers are reviewed. Finally, this presentation will describe initial outcomes and how this approach helps early diagnosis of HIV and notification among clients' sex partners. Seventy-one clients were referred by medical providers to project staff for an initial HIV knowledge and risk assessment and intensive health education. Data was collected from medical charts and STD/HIV records. Client's knowledge and subsequent STD infection was tracked to determine effectiveness of the health education component. Project screenings indicate patterns of high STD morbidity (especially syphilis), high numbers anonymous sex partners, and only 52% condom use at last sexual intercourse. Initial post-test findings reveal improvement of HIV transmission/treatment knowledge, improved condom negotiation skills, and 96% intent to use condoms with all sex partners. In conclusion, the combination of HIV risk reduction behavior intervention and epidemiologic contact investigation in a clinic may help reduce unprotected sex and the spread of HIV by known previous HIV positive persons.This project may show innovative use of existing resources to curb the spread of HIV/STDs.

672

MOBILITY IS A TRIGGERING FACTOR OF HIV/AIDS EPIDEMIC IN THE REGION OF KAYES, MALI

Modibo Keita[1], Yaya I. Coulibaly[2], Amadou Maiga[3], Brehima Bagayoko[1], Brehima Dicko[4], Hamidou A. Toure[5], Sitanfoune Veronique Coulibaly[6], Soumaila Keita[6], Oumar Guindo[7], Souleymane Ag Aboubacrine[8], Mahamdou M. Keita[9], Abdoulaye M. Traore[6], Dramane Sogoba[10], Abdel K. Traore[6], Sahare Fongoro[6], Sounkalo Dao[6]

[1]Hospital Fouseyni Daou of Kayes and CNAM, Bamako, Mali, [2]MRTC, Bamako, Mali, [3]Hospital Fouseyni Daou of Kayes, Kayes, Mali, [4]Reference Health Center of the District, Yelimane, Kayes, Mali, [5]Hospital Fouseyni Daou of Kayes, Bamako, Mali, [6]Hospital and University Center of Point G, Bamako, Mali, [7]Reference Health Center of the District of Commune IV, Bamako, Mali, [8]Ministry of Health Sectorial Cell for AIDS Control, Bamako, Mali, [9]Center for Vaccine Development/CNAM, Bamako, Mali, [10]SEREFO Research Project on HIV/TB, Bamako, Mali

The overall HIV prevalence rate is 0.7% in the region of Kayes -one of the lowest of the country. It represents the highest immigration area of Mali, West Africa. Due to these characteristics, we assessed the impact of the mobility of the population on the HIV/AIDS transmission pattern in the region. We defined by mobility any displacement of a resident of Kayes outside the country. We undertook a prospective study from January 2007 to December 2007 at the Regional Hospital Fousseyni DAOU of Kayes. A total of 109 HIV positive patients aged 14 years and above who have been found infected have been included. We compared patients having mobility history to those who never left the country according to vulnerability factors, attitudes, behaviors and practice towards HIV infection. Overall, 36.7% (40/109) of the patients had a mobility history. The frequency of that history was higher within the men 80.8% (25/31) as compared to the women 19.2% (15/78) (Fisher Exact test, $p<10^{-3}$). The notion of sexual intercourse during the mobility period has been reported by 67.5% of the 40 patients categorized as mobile. West Africa was the most frequent destination for these mobile subjects (57.5% of the cases). Mobile patients were more tolerant towards people living with HIV than those not having traveled. This attitude was illustrated by the fact they were significantly more susceptible to accept to share a meal with subjects infected with HIV even if they were not infected by the virus (OR=3.52; 95% CI= 1.48 - 8.37) or to work with them (OR=2.56; 95% CI= 1.082 - 6.075). Despite that positive attitude, mobile patients were 3 times more susceptible than the ones who never went abroad to have more than a sexual partner (OR=3.21; 95% CI= 1.37-7.57), to frequent a sex professional (OR=40.8; 95% CI= (5.12-325.3) and to have occasional partners (OR=31.12; 95% CI= 1.72-561.6). According to these data, the mobility in the region of Kayes, despite some positive attitudes towards social acceptation of people living with HIV, is an important factor of HIV expansion in the region of Kayes.

673

GENDER DIFFERENCES IN HEALTH LITERACY ABOUT TUBERCULOSIS (TB) AMONGST SOUTH AFRICAN HIGH SCHOOL STUDENTS

Myra Taylor

University of KwaZulu-Natal, Durban, South Africa

Health literacy, including modes of transmission, TB/HIV co-infection, prevention, signs/ symptoms and treatment, has a critical role in TB control through improved health-seeking behaviour, early diagnosis and adherence to TB treatment, and ultimately compliance with the NTB Programme recommendations. The objective of this study was to investigate health literacy about Tuberculosis amongst KwaZulu-Natal high school students. In a cross sectional study of 10 randomly selected KwaZulu-Natal urban/rural schools, students completed an anonymous semi-structured questionnaire investigating their knowledge, beliefs and attitudes, social support, self-efficacy, cues to action, intentions, and barriers to health seeking behaviour and treatment adherence, about TB using the Integrated Behaviour Change theoretical model. Of 1138 students, 98.0% isiZulu speaking (47.5% male, mean age 17.08 (SD 1.64) and 52.5% female, mean age 16.47 (SD (1.56), 36.5%, 32.4% and 31.1% were in grades 9, 10 and 11 respectively. Of these students 5.9% had previously received TB treatment. Although 69.2% of students considered TB to be a disease that usually affects the lungs, 54.7% females vs 44.3% males confirmed that TB can infect many part of the body (p=0.007). Significantly more females than males knew coughing >3 weeks to be a symptom of TB, that TB was not transmitted through using the same toilet as someone infected (41.9% vs 32.3%, p<0.005), perceived TB as treatable (45.7% vs 38.7%, p=0.006), knew that treatment takes 6 months (35.4% vs 25.8%), would encourage a TB patient to go to the clinic monthly (49.1% vs 41.6%, p=0.02), would remind to take TB tablets (48.1% vs 40.7%, p=0.005) and as a cue to action, knew someone cured of TB (30.2% vs 19.6%, p<0.005). However, fewer males (16.2% vs 20.1% believed that people with TB often get HIV). In conclusion, tuberculosis health literacy amongst KwaZulu-Natal high school students needs attention with a special focus on males to improve their health-seeking behaviour.

674

TREATMENT DEFAULT IS LOW AMONG PATIENTS INITIATING HAART AT THE KORLE-BU TEACHING HOSPITAL IN ACCRA, GHANA

April K. Wilhelm[1], Gabrielle Paci[1], Awewura Kwara[1], Margaret Lartey[2]

[1]Alpert Medical School of Brown University, Providence, RI, United States, [2]University of Ghana Medical School, Accra, Ghana

The expansion of highly active antiretroviral therapy (HAART) in the developing world has improved access to treatment and prognosis for HIV patients. An estimated 13,000 HIV-infected Ghanaians received HAART in 2007, of which Korle-Bu Teaching Hospital (KBTH) was a major provider. All KBTH HIV patients complete mandatory pre-treatment adherence counseling; however, it remains unclear how to further improve treatment outcomes in this setting. The objective of this study was to identify sub-groups of patients at-risk for treatment default in order to improve treatment outcomes among high-risk groups. We conducted a cross-sectional retrospective chart review of 290 HIV-infected patients who initiated HAART between January 1, 2008, and June 31, 2008. Demographics, clinical presentation, laboratory parameters, and treatment outcome data were collected from medical records. Chi-square and t-tests were used to compare demographic and clinical characteristics from patients continuing HAART and those who defaulted therapy. Of the 290 patients who initiated HAART, 242 (84%) continued on HAART, 41 (14%) defaulted, and 7 (2%) died during the 18-month study period. The mean±SD age was 38.7±9.4, 184 (64%) were female and 188 (66%) completed less than a secondary school education. The mean±SD baseline CD4 cell count was 183±144 cells/µl and body weight was 56±11 kg. Age, gender, educational level, marital status, presence or absence of opportunistic infection, BMI, baseline CD4 cell count, WHO disease stage 3 or 4, HAART initiation while pregnant, any incidence of poor adherence, and time to initiating HAART from clinic enrollment were not associated with treatment default ($P > 0.05$). A majority of patients initiating HAART in an urban Ghanaian clinic remained in care through one-year of follow-up. Patients who defaulted therapy were indistinguishable demographically and clinically from those who remained in care. Standardized pre-treatment adherence counseling sessions may have influenced the favorable outcomes.

675

IMMUNE RECONSTITUTION SYNDROME WITH HANSEN'S DISEASE IN A PATIENT WITH AIDS

Subramanian Swaminathan[1], Ooriapadikal Abraham[2], Susanne Abraham[2]

[1]Wayne State/Detroit Medical Center, Troy, MI, United States, [2]Christian Medical College, Vellore, India

A 35 year old man was diagnosed to have HIV infection, with a past history of cervical tuberculous lymphadenitis and esophageal candidiasis. His CD4 lymphocyte count was 50 cells/µl and HIV viral load 266370 copies/ml. Therapy was started with anti retroviral drugs with stavudine, lamivudine and nevirapine. Fifteen days after starting therapy, examination revealed mildly edematous and erythematous plaques on his trunk and extremities, and non tender enlargement of the common peroneal nerves. Skin smears from one of the plaques revealed borderline tuberculoid leprosy with type I reaction. CD4 lymphocyte count at 2 months of anti retroviral therapy (ART) was 112 cells/ µl and viral load was undetectable (<400 copies/ml). Therapy was started for Hansen's disease with chloroquine along with a tapering dose of prednisone. The skin lesions improved markedly with therapy, and became smear negative on follow up. He completed 2 years of therapy and has remained well without relapses on further follow up for 5 years. Our patient manifested with features of Hansen's disease as an immune reconstitution syndrome after initiation of ART for AIDS with prompt response to therapy. New skin lesions after starting ART should trigger performance of skin smears for Hansen's disease in endemic areas.

676

PNEUMOCYSTIS JIROVECII IN SUB-SAHARAN AFRICA: LOW PREVALENCE OF LUNG COLONIZATION IN UGANDAN AIDS PATIENTS WITH NON-PNEUMOCYSTIS PNEUMONIA

Steve M. Taylor[1], Steven R. Meshnick[1], William Worodria[2], Alfred Andama[2], J. Lucian Davis[3], Adithya Cattamanchi[3], Saskia den Boon[2], Samuel Yoo[2], Laurence Huang[3]

[1]University of North Carolina Gillings School of Global Public Health, Chapel Hill, NC, United States, [2]Makerere University-University of California, San Francisco Research Collaboration, Kampala, Uganda, [3]University of California, San Francisco, San Francisco, CA, United States

Pneumocystis pneumonia (PcP) is a common opportunistic infection in patients with AIDS in the developed world, but the burden of PcP in sub-Saharan Africa in unknown. *Pneumocystis jirovecii* colonization can provide insight into the epidemiology and biology of the fungus. We assessed the prevalence of *P. jirovecii* colonization in respiratory specimens from consecutive HIV-positive patients with cough ≥ 2 weeks who were admitted to Mulago Hospital in Kampala, had negative sputum acid-fast bacillus smears, and underwent bronchoscopy; all samples were Diff-Quik (modified Giemsa) stain-negative, and were tested with a nested PCR assay targeting *P. jirovecii* mitochondrial rRNA. 124 patients were enrolled from September 2007 to July 2008. The median CD4 cell count was 88 cells/mm3 (IQR 22 - 196), and for 31 (25%) patients HIV was previously undiagnosed. Of the 93 patients with known HIV infection, 77 (83%) reported taking either TMP/SMX (n=75) or dapsone (n=2) to prevent PcP. Ultimate clinical diagnoses were bacterial pneumonia in 68 (55%), pulmonary tuberculosis in 37 (30%), and other or unknown diagnoses in 19 (15%). The prevalence of *P. jirovecii* colonization was 6% (7/124). In 93 patients with known HIV infection, 5 (5%) were colonized, all of whom reported taking TMP/SMX; among 29 patients with previously-undiagnosed HIV, 2 (7%) were colonized. The median CD4 count was lower in colonized (58) than non-colonized patients (91; p=0.47). During followup, 5 of 7 colonized patients died (71%), compared with 29 of 117 non-colonized patients (25%; p<0.01); controlling for CD4 count, clinical diagnosis, ARV and prophylactic antibiotic receipt, and age, *P. jirovecii* colonization was independently associated with death (OR 16; 95% C.I. 1.42 - 177). Sequencing of the mitochondrial DNA target suggested multiple *P. jirovecii* strains in study patients. In contrast to reports from the developed world, the prevalence of *P. jirovecii* colonization is low in hospitalized HIV-positive patients in Kampala. Its strong association with death during followup merits further inquiry.

677

BEST PRACTICES IMPLEMENTED AT A LOCAL ART CENTER IN GUJARAT, INDIA

Katya Calvo[1], Ravi Durvasula[1], Pratab Shah[1], Arti Trivedi[2]

[1]University of New Mexico, Albuquerque, NM, United States, [2]Pandit Deendayal Upadhyay Medical College, Rajkot, India

In resource limited settings, the percentage of HIV patients on anti-retroviral therapy (ART) that are lost to follow-up (LFU) ranges between 8-21%. In India, the National AIDS Control Organization (NACO) has 217 clinics nationwide, with an average LFU of 7%. In the state of Gujarat, a local ART center in Rajkot has a LFU percentage of 2.4%, while maintaining a record of more than 95% adherence in 92 percent of patients on ART. The objective of this study was to observe and record clinic-based best practices implemented that decrease LFU and increase adherence. Observation and recording of day-to-day clinical practices was utilized. Best practices implemented at ART clinic include: aggressive pre-appointment outreach including phone calls and text messaging, rapid re-scheduling for missed appointments, multiple sessions with counselors for education on HIV and ART therapy during a single clinic visit, questionnaire given to patient assess level of understanding after counseling, session with pharmacist at every visit, providing allowance for meals for patients on ART, Community Care Centers, and Linked Centers. In conclusion, the implementation of several clinic-based best practices has decreased the LFU rate to 2.4% in an ART center in a resource limited country

678

MALARIA PIGMENTS: A SIGNATURE OF IMPAIRMENT OF PHAGOCYTOSIS IN HIV AND MALARIA CO-INFECTION

Alex Ogwal

Infectious Diseases Institute, Kampala, Uganda

Phagocytosis by monocytes and neutrophils has long been recognized to play a crucial role in the defense of the host against opportunistic infections including malaria. We hypothesized that HIV-infection might impair phagocytosis by peripheral monocytes and neutrophils predisposing infected patients to frequent bacterial and fungal infections. To test this hypothesis, we assessed the proportion of phagocytes containing malaria pigments as a phagocytosis outcome in HIV-infected and uninfected patients. Giemsa stained peripheral blood smears were microscopically examined for phagocytes containing malaria pigments in 101 patients, consisting of 50 patients presenting with HIV-1 and malaria co-infection; and 51 HIV-negative patients presenting with malaria infection alone. The proportion of phagocytes containing malaria pigments were higher in HIV-negative patients (69.8%) than in HIV-infected patients (30.2%) (p< 0.0001). HIV-infected patients were four times more likely to have impaired phagocytosis (Odds ratios (OR) = 4.4, 95% CI=1.9-10.3) than HIV-negative patients. There was no significant difference in malaria parasitemia between the two groups. (p< 0.46). In conclusion, HIV infection may impair phagocytosis. The non significant difference in parasitemia could be due to the usage of cotrimoxazole by HIV infected patients. Functional improvement of phagocytosis may lead to better disease outcome in HIV-infected patients.

679

BIOMARKERS OF MORBIDITY ASSOCIATED TO HTLV-1 INFECTION

Edgar M. Carvalho[1], Paulo Oliveira[1], Marina Caskey[2], Andre Muniz[1], Silvane Santos[1], Glória Orge[1], Matheus T. Santos[1], Davi Tanajura[1], Marshall Glesby[2], Neviton Castro[1]

[1]Federal University of Bahia, Salvador, Brazil, [2]Cornell University Medical College, New York, NY, United States

The human T cell lymphotropic vírus type 1 (HTLV-1) infection is a neglected disease because morbidity associated to this virus infection is considered to be low. In fact HTLV-1 associated mielopathy or tropical spastic paraparesis (HAM/TSP), the main disease associated to HTLV-1, only occurs in less than 2% of the cases. The objective of this study is to demonstrate that a high percentage of HTLV-1 infected individuals have clinical and neurological manifestations and to determine biomarkers of expression of neurologic disease in HTLV-1. In addition to clinical, dentistry and neurological examination, determination of cytokines TNF-α, IFN-γ and IL-17 were performed in supernates of unstimulated mononuclear cell cultures. A cross sectional study comparing manifestations in 115 HTLV-1 carriers and 115 seronegative blood bank donors showed that dry mouth, periodontitis, arthritis, foot numbness, weakness, urinary manifestations and erectile dysfunction (ED) were significantly more frequent in HTLV-1 than in controls. A cross sectional study was also performed in 105 males divided in 3 groups: 1) HAM/TSP; 2) HTLV-1 infected subjects with neurological complains but who do not fulfill the criteria for HAM/TSP and 3) asymptomatic HTLV-1 carriers. ED and or overactive bladder (OB) was observed in all patients with HAM/TSP, in 67% of patients in group 2 and in 35% of patients of group 3. Moderate and severe ED was observed in a large percentage of these individuals and there was no association between ED and age. OB was highly associated with ED (P<.001). While there was no difference between IFN-γ and TNF-α levels and proviral load between patients with OB and HAM/TSP, these markers were significantly increased in individuals with OB in comparison to asymptomatic HTLV-1 carriers. These data indicate that a large percentage of HTLV-1 infected individuals who do not fulfill the criteria for HAM/TSP have already evidence of ED and OB. Additionally proviral load and increasing in pro-inflammatory cytokines are biological markers of neurological damage in HTLV-1 infection.

680

DISSEMINATED LEISHMANIASIS WITH INTESTINAL LESIONS IN AN HIV PATIENT

Sadia Zahid, Shadab Ahmed, Wondwoosen Gebre, Kaleem Rizvon, Rekha Nagwekar, Gurpreet Bambra, Tabassum Yasmin, Janice Verley, Hesham M. Taha

Nassau University Medical Center, East Meadow, NY, United States

Leishmaniasis is a protozoal disease transmitted by sandfly vectors and is endemic in South America, Asia, Africa, southern Europe, and Mediterranean countries. 45 years old male immigrant from Guatemala was admitted with ataxia and pancytopenia. The patient had HIV/AIDS with CD4 count 60/mm3 and HIV-1RNA 338cps/ml on ARV therapy. On examination, he weighed 54kg, was cachectic with marked hepato-splenomegaly and no lymphadenopathy. CT revealed the liver measuring 17 x 14 x 23 cm and the spleen measuring 25 x 21 x 11 cm. He had WBC of 1100/mm3, Hb 6.6g/dL, Hct 19.5, and platelets 99000/mm3. He underwent EGD and colonoscopy. Biopsy of the small bowel, gastric antrum, and rectum showed predominant histiocytes with amastigotes in lamina propria and mucosa consistent with *Leishmania*. These slides were sent to Centers for Disease Control and Prevention, Atlanta where diagnosis was confirmed. The Indirect Fluorescent Antibody titre for *L. donovani* was ≥ 1:256. Bone marrow aspirate, sent to Wadsworth Labs, NYSDOH actually grew *Leishmania* identified as species *donovani.* Stains and serology for *Toxoplasma*, *Histoplamsa*, and *Cytomegalovirus* were negative. Treatment was given with Liposomal Amphotericin B (Ambisome®) at 4mg/kg for 7 days followed by once weekly dosing for 5 weeks. After completing one cycle of the treatment, he had repeat biopsies which showed elimination of the organisms from most of the gut except parts of small intestine. Also there was significant decrease in his hepato-splenomegaly. Patient is undergoing 2nd cycle of treatment with Ambisome® at 5mg/kg. Leishmaniasis is rare in the United States but has been reported in areas bordering Mexico such as rural southern Texas. The most common forms are cutaneous leishmaniasis, causing skin sores; and visceral leishmaniasis, which affects spleen, liver, and bone marrow. Disseminated leishmaniasis in HIV/AIDS with such extensive GI tract involvement especially involving the stomach, small & large intestine and rectum; besides liver, spleen, and bone marrow, as seen in our patient is rare. It is considered an opportunistic pathogen in immunosuppressed patients with a high relapse rate after treatment, thus necessitating perhaps, secondary prophylaxis. There is no definite guideline available for treatment failure or relapse. We recommend a subsequent cycle of therapy and maintenance dosing as secondary prophylaxis.

681

SEROPREVALENCE OF *LEISHMANIA INFANTUM* IN DOGS FROM KOREA

Se Eun Choe, Seung Won Kang, Chang Hee Kweon, Suk Chan Jung

National Veterinary Research and Quarantine Service, Anyang, Republic of Korea

It has been reported that clinically infected dogs were the main reservoir hosts of visceral leishmaniasis (VL) caused by *Leishmania*. Leishmaniasis has never been reported in Korea. Many techniques have been used for diagnosis of VL, among which latex agglutination test (LAT) is known to be simple and applicable to all species. The objective of this study was to assess the prevalence of *L. infantum* antibodies in dogs from Korea using LAT. To achieve this, LAT was standardized with 8 positive control samples obtained from Italy and the test result was compared with that of commercial ELISA. The Kappa value (κ) was used to evaluate the level of agreement between LAT and ELISA. Strength of agreement based on κ was judged according to the following guideline: <1.45= poor, 0.45-0.75= good, >0.75= excellent. The level of agreement between LAT and commercial ELISA for diagnosis of VL was found to be 0.73 (serum dilution rate 1:32) and 1(serum dilution rate 1:64). A total of 332 serum samples collected from dogs in Korea were tested by the LAT using a 1: 64 titer as positive cut-off, and all sera were negative for *L. infantum* antibodies. In this study, we report for the first time the result of serological survey of *L. infantum* in dogs in Korea. Also, the LAT standardized in this study yielded a satisfactory agreement with ELISA, indicating it can be recommended as a rapid, field applicable and reliable test for diagnosis of VL.

682

DIHYDROQUINOLINES WITH *IN VITRO* AND *IN VIVO* ACTIVITY AGAINST AFRICAN TRYPANOSOMES

Carolyn Reid[1], Shanshan He[1], Donald A. Patrick[2], Premalatha Kokku[1], Jean Fotie[1], Tanja Wenzler[3], Reto Brun[3], Richard R. Tidwell[2], **Karl Werbovetz**[1]

[1]The Ohio State University, Columbus, OH, United States, [2]University of North Carolina, Chapel Hill, NC, United States, [3]Swiss Tropical and Public Health Institute, Basel, Switzerland

Dihydroquinolines showed selective *in vitro* activity against *Trypanosoma brucei rhodesiense* STIB900, and the lead compound OSU-40 (1-benzyl-1,2-dihydro-2,2,4-trimethylquinolin-6-yl acetate) displayed efficacy in the *T. b. brucei* STIB795 murine trypanosomiasis model (as reported previously). These compounds are believed to act as prodrugs which are converted to active dihydroquinolin-6-ols. The dihydroquinolin-6-ols may exert their trypanocidal effects through redox cycling in the parasite. Subsequent studies sought to further define the antitrypanosomal structure-activity relationship of this series of molecules and to

examine the stability and antitrypanosomal activity of dihydroquinolin-6-ol hydrochloride salts. Placement of substituents at the 3-, 4- and 8-positions of the 6-acetoxydihydroquinoline core dramatically reduced antitrypanosomal activity compared to OSU-40. The addition of a phenyl ring at the 7-position also abolished activity against *T. brucei*. However, activity was maintained when a small substituent was placed at the 7-position. We also found that a prodrug approach is not required; dihydroquinolin-6-ol hydrochloride salts are stable crystalline materials that display nanomolar *in vitro* antitrypanosomal activity. Both 6-acetoxydihydroquinoline prodrugs and dihydroquinolin-6-ol hydrochloride salts produced cures in the *T. b. rhodesiense* STIB900 murine trypanosomiasis model. OSU-75 (1-(2-methoxybenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-yl acetate) and OSU-95 (1-(2-methoxybenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-ol hydrochloride) cured infected mice when these compounds were administered i.p. for 4 days at 50 mg/kg/day starting the day after infection.

683

DEVELOPMENT OF PK-PD MODELS TO PREDICT THE THERAPEUTIC DOSE AND CNS DISPOSITION OF SCYX-7158 IN THE TREATMENT OF STAGE 2 HUMAN AFRICAN TRYPANOSOMIASIS

Stephen Wring[1], Eric Gaukel[1], Bakela Nare[1], Robert Jacobs[1], Cy Bacchi[2], Beth Beaudet[1], Tana Bowling[1], Daitao Chen[1], Yvonne Freund[3], Matthew Jenks[1], Luke Mercer[1], Andy Noe[1], Matt Orr[1], Robin Parham[1], Jacob Plattner[3], Ryan Randolph[1], Cindy Rewerts[1], Jessica Sligar[1], Nigel Yarlett[2], Robert Don[4]

[1]Scynexis Inc., Research Triangle Park, NC, United States, [2]Pace University, New York, NY, United States, [3]Anacor Pharmaceuticals Inc., Palo Alto, CA, United States, [4]DNDi, Geneva, Switzerland

SCYX-7158 is a potent trypanocidal oxaborole-6-carboxamide that DNDi is currently progressing through formal pre-clinical safety studies with the goal of becoming the first oral treatment for Stage 2 (neurological or CNS) Human African Trypanosomiasis (HAT). SCYX-7158 achieves 100% cures in a murine Stage 2 model of HAT after 7 daily oral 25mg/kg doses. Efficacy correlates with SCYX-7158 exposure (concentration and time) in brain tissue. This work presents PK and tissue distribution data in rodents, dogs and non-human primates, with *in vitro* time-kill data, to develop predictive models of (i) SCYX-7158 concentrations in brain and CSF from plasma concentrations and (ii) efficacious dose in rodents. The importance of PK and *in vitro* DMPK data in building the PK-PD models will be discussed. SCYX-7158 is highly permeable in an *in vitro* MDCK-MDR1 model of the blood-brain barrier (Papp >400nm/s) and is not a substrate for the P-gp efflux transporter (absorption quotient <0.1), suggesting it should readily enter the CNS. Binding to plasma proteins was concentration dependent, and with binding to brain tissue proved most important for influencing CNS disposition. The unbound fraction (fu) of SCYX-7158 in mouse plasma at the MIC (~0.6µg/mL) was 0.3% rising to 3.2% at plasma concentrations equivalent to Cmax at steady-state (~15µg/mL, 25mg/kg doses). Binding to brain tissue was independent of concentration (fu(brain) ~5%). Plasma and brain unbound fractions were determined for 3 additional oxaboroles that have demonstrated efficacy in either Stage 1 (hemolymphatic) but not Stage 2, or both Stage 1 and 2 murine HAT models. Including SCYX-7158, the log D range was 3.8-4.6 corresponding to fu(plasma) 3.3-0.35%, and fu(brain) 17.7-5.4% (at 2µM). These data supported the hypothesis that CNS disposition and hence efficacy in the Stage 2 model is driven by the balance of binding to plasma proteins and brain tissue. We are currently extending this work to develop allometric scaling parameters to model CNS exposure and predict an efficacious dose for clinical studies.

684

SEVERE ORGAN AND TISSUE ABNORMALITIES IN ANKOLE CATTLE EXPERIMENTALLY INFECTED WITH *TRYPANOSOMA BRUCEI BRUCEI*

Ann Nanteza[1], Zachariah Nsadha[1], Sam Eyanu[1], Monica Namayanja[1], Rosemary Flores[2], Elisabeth Knapp[2], Roger Prichard[3], Vidadi Yusibov[2], George W. Lubega[1]

[1]Department for Veterinary Parasitology and Microbiology, Makerere University, Kampala, Uganda, [2]Fraunhofer USA Inc., Center for Molecular Biotechnology, Newark, DE, United States, [3]Institute of Parasitology, McGill University, Montreal, QC, Canada

In Sub-Saharan Africa, trypanosome infection causes serious diseases in humans (sleeping sickness) and in animals (nagana). The acute disease in cattle is believed to be caused by *Trypanosoma congolence* and *T. vivax*, whereas *T. b. brucei* is considered to be a mild pathogen. However, conclusive evidence that *T. b. brucei* is not a significant pathogen in cattle is obscured as it often occurs in mixed infections with *T. congolense* and *T. vivax*. To this end, we undertook controlled indoor infection studies in Ankole Longhorn breed calves with a *T. b. brucei* strain, which was isolated from naturally infected Ugandan cattle. All infectious doses tested were lethal to the Ankole Longhorn breed calves. We monitored disease progression using conventional parameters such as parasitemia and packed cell volume. We performed extensive post-mortem examinations on the diseased cattle throughout the progressing stages of the disease to examine tissue-invasiveness of this pathogenic *T. b. brucei* strain. Detailed histological analysis revealed accumulation of trypanosomes in different organs. We observed organ abnormalities and severe lesions in sacrificed animals even though the parasites were absent from the bloodstream during the entire chronic phase of the disease. We suggest that the inflammatory and degenerative tissue changes observed might, at least partially, be due to mononuclear cell infiltration. Our findings substantiate that *T. b. brucei* differs from *T. congolense* and *T. vivax*, which are known to be confined to the vascular system. Our data suggest that the role of *T. b. brucei* as a pathogen might have been underestimated in the past.

685

DISCOVERY AND OPTIMIZATION OF A SERIES OF BORON-CONTAINING SMALL MOLECULES AS POTENTIAL DRUG CANDIDATES FOR THE TREATMENT OF HUMAN AFRICAN TRYPANOSOMIASIS

Matthew Orr[1], Eric Gaukel[1], Matthew Jenks[1], Bakela Nare[1], Cindy Rewerts[1], Stephen Wring[1], Charles Ding[2], Yvonne Freund[2], Jacob Plattner[2], Cyrus Bacchi[3], Nigel Yarlett[3], Robert Don[4], Robert Jacobs[1]

[1]SCYNEXIS, Inc., Durham, NC, United States, [2]Anacor Pharmaceuticals, Inc., Palo Alto, CA, United States, [3]Pace University, New York, NY, United States, [4]Drugs for Neglected Disease Initiative, Geneva, Switzerland

Human African Trypanosomiasis (HAT) represents a significant public health problem in sub-Saharan Africa affecting hundreds of thousands of individuals. An urgent need exists for the discovery and development of new, safe, and effective drugs to treat HAT, as existing therapies suffer from poor safety profiles, difficult treatment regimens, limited effectiveness, and a high cost of goods. From a collaborative effort between SCYNEXIS, Anacor Pharmaceuticals, Pace University, and DNDi, we report the discovery and lead optimization of a novel class of boron-containing small molecules. These compounds inhibit *in vitro* growth of *T. brucei* with sub-micromolar IC50's, show no cytotoxicity to mammalian cells, and exhibit good physiochemical and pharmacokinetic properties. Development of a structure-activity relationship (SAR) profile for this chemical series and efforts to improve biological and pharmacokinetic profiles through chemical modifications will be described.

686

IDENTIFICATION OF POTENTIAL DRUG TARGETS IN PROTOZOAN PARASITES USING COMPARATIVE GENOMICS

Barbara Nerima[1], Pascal Maeser[2]

[1]National Livestock Resources Research Institute, Tororo, Uganda, [2]Swiss Tropical and Public Health Institute, Basel, Switzerland

Protozoan parasites are unicellular organisms responsible for many diseases like Malaria, Trypanosomiasis, Chagas disease, Leshimanioses, etc. Management of such diseases relies heavily on accurate diagnosis and effective chemotherapy. However, for many of the diseases chemotherapy relies on a few drugs that are ineffective, highly toxic and expensive. There is therefore need to identify new drug targets for which drugs can be developed. With the availability of fully sequenced genomes, potential drug targets can be identified by comparative genomics. The advantage with this approach is that a drug`s target is known in advance thus providing a rational base for drug combinations. Parasites have lost many metabolic reactions or pathways in the course of evolution. However, they have also retained certain reactions that are missing in the host and therefore can be targeted by drugs. In this work we reconstructed and compared core metabolism of free-living organisms and obligate parasites using the available genome information and metabolic pathway databases. Several enzymes were found to be specific to parasites and hence potential drug targets. For example salvage of methionine in *Trypanosoma brucei* is catalysed by methionine synthase (EC2.1.1.14) and homocysteine methyltransferase (EC2.1.1.10), both of which are absent in the human host. We investigated the importance of such parasite-specific enzymes by reverse genetics and found them to be essential to the parasite at Physiological concentrations of methionine and hence potential drug targets.

687

DEVELOPMENT OF A MEDIUM THROUGHPUT SYSTEM THAT USES LYMPH NODE *EX VIVO* EXPLANT CULTURES TO IDENTIFY COMPOUNDS AGAINST CUTANEOUS LEISHMANIASIS

Alex G. Peniche[1], Elvia Y. Osorio[1], Peter C. Melby[2], Bruno L. Travi[1]

[1]University of Texas Health Science Center at San Antonio, San Antonio, TX, United States, [2]University of Texas Health Science Center at San Antonio/South Texas Veterans Health Care System, San Antonio, TX, United States

Cutaneous leishmaniasis affect >10 million people in endemic regions, worldwide. New drugs are needed because current therapies are toxic, expensive, and their efficacy is hindered by parasite resistance. Golden hamsters and Balb/c mice are susceptible to *Leishmania Viannia spp*. and *L. major*, respectively, and provide good models for testing anti-Leishmania drug candidates. We developed a model system to screen for anti-Leishmania compounds that utilizes the intracellular amastigote form of the parasite, including other cell populations involved in the host's immune response. The system uses *Leishmania* transfected with an episomal vector containing the luciferase reporter gene, which facilitates parasite quantification compared with traditional microscopy. The animals were inoculated intradermally in the snout and ears with stationary phase infective promastigotes selected by incubation with hamster Complement (*L. panamensis*) or concentration of metacyclic forms using peanut agglutinin (*L. major*). The cervical lymph nodes were used as sources of lymphocytes and infected macrophages to test compounds in the *ex vivo* system. The evaluation of parasite burden by means of luminometry showed that at 28 days p.i. the parasite load was adequate (>100 photons/sec for 1.2-1.5x104 cells) for evaluating the leishmanicidal efficacy of drugs in a medium throughput format. Using an established *in vitro* therapeutic index >5, a varying proportion of 54 lead compounds that were previously identified as active against *L. donovani* showed to have activity against *L. major* (87%) and *L. panamensis* (35%). Among them, Disulfiram, which inhibits acetaldehyde dehydrogenase in humans, has shown promising results as topical treatment for both species of cutaneous leishmanias (>90 fold decrease in lesion parasite burden) and different therapeutical regimes are currently under evaluation in animal models.

688

DISCOVERY OF CYSTEINE PROTEASE INHIBITORS WITH ANTI-*TRYPANOSOMA CRUZI* ACTIVITY

Momar Ndao[1], Deborah Nicoll-Griffith[2], Christian Beaulieu[2], Cameron Black[2], Elise Isabel[2], Fabio Vasquez Carmago[1], Frédéric Massé[2], Christophe Mellon[2], Nathalie Méthot[2], Doris Lee[2], Hyeram Park[2], Milli Nath Chowdhury[1]

[1]McGill University, Montreal, QC, Canada, [2]Merck Frosst Research Centre, Kirkland, QC, Canada

Chagas disease which is estimated to affect some 16 million people in South America is caused by the parasite *Trypanosoma cruzi*. There are currently no FDA approved therapies available to treat the infection. The cysteine protease cruzipain is involved in all stages of the development of the parasite *T. cruzi*. This parasite is responsible for the propagation of Chagas disease. An *in vivo* POC study in mice demonstrated the efficacy of the basic cruzipain inhibitor Cz008 to significantly reduce the number of parasites in mice infected with the Brazilian strain of *T. cruzi*. However the basic nature of Cz008 is likely associated with lysosomotropism in which the accumulation of the compound in lysosomes could induce a loss of selectivity over off-target cathepsins such as Cat F, L and S. This prompted us to develop the non-basic inhibitor of cruzipain Cz007 for *in vivo* testing. A second study in *T. cruzi*-infected mice was conducted and showed that Cz007 was equipotent to Cz008, demonstrating that a basic inhibitor is not required for *in vivo* efficacy. The inverse dose response observed for Cz007 may be attributed to inhibition of off-target cathepsins at higher doses of this poorly selective inhibitor. A major breakthrough in intrinsic selectivity of these non-basic compounds was obtained with the replacement of the fluoroleucine in P2 by valine to afford a potent and selective inhibitor. Further SAR around P-1 and P-3 portions led to the identification of Cz009 which has suitable potency, selectivity and pharmacokinetics for further development.

689

DEVELOPMENT OF A REAL-TIME POLYMERASE CHAIN REACTION ASSAY FOR IDENTIFICATION OF THE CAUSAL AGENTS OF LEISHMANIASIS IN PERU

Jorge Nunez[1], Maxy De los Santos[1], Pablo Tsukayama[1], Valeria Soberon[1], Braulio Valencia[2], Allejandro Llanos-Cuentas[2], David J. Bacon[1], Paul C. Graf[1]

[1]Naval Medical Research Center Detachment, Lima, Peru, [2]Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru

In Peru, several species of *Leishmania* (*Viannia*) and *Leishmania* (*Leishmania*) are responsible for cutaneous and mucocutaneous leishmaniasis. The gold standard for species identification is multilocus enzyme electrophoresis (MLEE), a time-consuming method that also requires successful culture of the parasites from the leishmaniasis lesion on the patient. Based on sequence polymorphisms in the mannose phosphate isomerase (MPI) and 6-phosphogluconate dehydrogenase (6PGD) genes, used for MLEE, we have developed a new real-time polymerase chain reaction (RT-PCR) that combines fluorescence resonance energy transfer (FRET) and melting curve analysis. This assay allows discrimination among closely related species, specifically *L.* (*V.*) *braziliensis* from *L.* (*V.*) *peruviana* and *L.* (*V.*) *guyanensis* from *L.* (*V.*) *panamensis*, directly from clinical samples. One hundred seventeen biopsies and fourteen lancet scrapings were tested in our assay. Samples were also assayed by conventional *Leishmania* diagnostic tests including intra-dermal reaction (Montenegro test), microscopy and parasite culture. More than 90% of the cases were diagnosed as *L.* (*V.*) *peruviana*, *L.* (*V.*) *braziliensis*, and *L.* (*V.*) *guyanensis*,

the three most common species present in Peru. Four biopsy samples from Ecuador were also tested and diagnosed as *L.* (*V.*) *panamensis*. The sensitivity of the molecular assay was of 85% and 70% for biopsies and lancet scrapings, respectively, as compared to 20% to 70% for the traditional diagnostic methods. The RT-PCR assay is a sensitive and rapid alternative that could be incorporated as an additional diagnostic method of leishmaniasis in reference centers in Peru.

690

HAND-HELD EXO-CRYSTALLIZATION THERMOTHERAPY AS A PROMISING ALTERNATIVE FOR AMERICAN CUTANEOUS LEISHMANIASIS

Braulio M. Valencia[1], David A. Miller[2], Richard Witzig[3], Alejandro Llanos-Cuentas[1]

[1]Institute of Tropical Medicine Alexander von Humboldt, Lima, Peru, [2]University of Chicago, Department of Internal Medicine, Chicago, IL, United States, [3]Infectious Diseases Section, Tulane School of Medicine, New Orleans, LA, United States

American Cutaneous leishmaniasis (CL) is characterized by variable cure rates and reduced therapeutic alternatives. Pentavalent antimonials (Sb5+) are associated with several side effects and clinical resistance is now more frequently reported. Hand-held Exo-Crystallization thermotherapy (HECT-CL) is based in heat released due to sodium acetate crystallization reaching control and maintained temperatures of 51 +/- 1ºC for at least 5 minutes. Patients with confirmed CL (scraping, culture or PCR) with/ without prior therapy were treated with 5-7 days of HECT-CL alone or in combination with topical Imiquimod (IMQ). HECT-CL was applied daily during 3 minutes continuously or divides 2 or 3 times depending of tolerability. These patients received HECT-CL as a compassionate treatment due to pregnancy, exclusive breastfeeding, cardiac contraindications, or inability to receive the only second line treatment recognize in our country (Amphotericin B). Patients were continuously evaluated during the 5-7 days of treatment to identify second-degree burns or super-infection. Close follow-up was performed during 6 months to identify early failure and start a new-therapeutic regimen. 9 patients were included; 6 were male, mean age was 23.6 years (SD: 18) and 8 of them received previous treatment. Only one patient was treatment naïve and received HECT-CL due to exclusive breastfeeding. All patients received Sb5+ like previous treatment and 2 of them were children who received 2 courses of Sb5+ and required Amphotericin B like a second-line alternative. 8 presented the recurring form of Leishmaniasis recivida cutis (LRC, small nodules over or close to the prior atrophic scar) and the breastfeeding patient presented 2 nodular lesions. All patients received HECT-CL with a duration illness (or reactivation) of less than 4 weeks. 4 patients cured (HECT-CL+IMQ), one presented reactivation during the third month of follow-up (HECT-CL+IMQ) and the remaining 4 are actually in follow-up without signs of relapse (2 with HECT-CL+IMQ and 2 with HECT-CL alone). In conclusion, HECT-CL is a safe alternative for recurrent and new cases of CL. Its use can be extended to special cases of CL during pregnancy, breastfeeding, people with cardiac contraindications and children. It seems to be useful for treatment of early lesions without increase risks of failure.

691

TUBULIN-BASED VACCINE CANDIDATES TO COMBAT AFRICAN ANIMAL TRYPANOSOMIASIS

Elisabeth Knapp[1], Lauren Goldschmidt[1], Rosemary Flores[1], Thomas Kraemer[1], Monica Namayanja[2], Ann Nanteza[2], Jessica Chichester[1], Tatiana Golovina[1], Roger Prichard[3], George W. Lubega[2], Douglas Holtzman[4], Vidadi Yusibov[1]

[1]Fraunhofer USA Inc., Center for Molecular Biotechnology, Newark, DE, United States, [2]Department for Veterinary Parasitology and Microbiology, Makerere University, Kampala, Uganda, [3]Institute of Parasitology, McGill University, Montreal, QC, Canada, [4]The Bill and Melinda Gates Foundation, Seattle, WA, United States

African trypanosomiasis is fatal to humans and animals if left untreated. The disease poses a serious threat to public health and causes enormous economic losses in sub-Saharan Africa. Despite decades of efforts, no effective vaccine has been developed against this disease, because the trypanosome continuously changes the dominant variable surface glycoprotein (VSG) antigens that cover nearly the entire surface of the parasite, making it inappropriate for vaccine development. To overcome this obstacle, we identified non-variable antigens of the parasite that can generate protective immunity. Tubulin, one such candidate, was shown to confer protection in mice when animals were challenged with homologous or heterologous strains of *Trypanosoma*. We have engineered regions of α and β-tubulin of *Trypanosoma brucei* as fusions with the coat protein of a plant virus, *Alfalfa mosaic virus* (AlMV) and produced them as virus-like particles. Plant-produced recombinant AlMV particles displaying target peptides from α or β tubulin stimulated protective immune responses in animals. Current research is dedicated towards understanding the mode of protection.

692

EFFECTS OF IMMUNOSUPPRESSION IN THE EXACERBATION OF PARASITISM BY *TRYPANOSOMA CRUZI* IN MICE WITH ACUTE CHAGASIC INFECTION

Maritza Alarcon, Claudia Ruiz-Agelvis, Ana Lugo-Yarbuh, Elio Moreno, Sonia Araujo

Universidad de los Andes, Merida, Bolivarian Republic of Venezuela

Chagas disease is caused by *Trypanosoma cruzi,* described by Carlos Chagas in Brazil, 1909. This disease has a wide distribution in Latin America with high levels of prevalence and severity of clinical pictures. One of the lines of research into this disease is based on the histopathology. In this study, 12 mice male NMRI were injected intraperitoneally with 2x104 bloodstreams of M/HOM/BRA/53/Y *T. cruzi* strain. At day 5 of infection, the mice were immunosuppressed with 0.05 mL of Endoxan, receiving a second dose two days later. The patent parasitemia increased in infected mice immunosuppressed, between 5 and 15 days post-infection, compared with the mice not immunosuppressed and with significant differences ($P<0.05$). At 15 days pi, mice were sacrificed, and the heart and skeletal muscle were removed for evaluate *T. cruzi* infection. The tissue were fixed in formalin to 10%, included in paraffin and staining with Hematoxylin and Eosin. The histopathological study revealed nests of amastigotes, inflammatory infiltrate with mononuclear and polymorphonuclear cells, destruction of skeletal muscle fibres and cardiac tissue with myositis and myocarditis. The results demonstrated an exacerbation of the parasitism in infected mice, as result of the immunosuppressive chemical agent activity during the course of infection by *T. cruzi.* It might be related with the reduction of the activity on the immune system of infected mice immunosuppressed compared with control mice. These findings can be extrapolated to human cases with Chagas´disease either medical treatment immunosuppressive or with other clinical conditions.

693

MATERNAL INFECTION BY *TRYPANOSOMA CRUZI* INDUCES AN CELLULAR IMMUNE RESPONSE WITH CYTOKINES PRODUCTION IN FETUSES

Maritza E. Alarcon, Mary C. Perez-Aguilar, Loredana Goncalves, Sonia Araujo, Elio A. Moreno

Universidad de los Andes, Merida, Bolivarian Republic of Venezuela

To detect the cytokines IFN-γ, IL-4 and IL-10 expressed by CD4 + T cells in tissues of fetal mice with acute chagasic infection. Examined the fetuses of NMRI mice infected with 22x103 trypomastigotes metacyclic the strain M/HOM/BRA/53/Y of *T. cruzi* and pregnant during the acute phase of infection, for the detection and localization of inflammatory infiltrates, nest parasites, remains antigenic and cytokines, we used hematoxylin-eosin techniques, peroxidase-anti-peroxidase and immunofluorescence. The study immunohistochemistry revealed the presence of abundant inflammatory infiltrate and antigenic deposit with amastigotes nests in fetal skeletal muscle. The detection of IFN-γ, IL-4 and IL-10 was carried out in the placenta, heart and skeletal muscle fetal using CD4+ and CD4- cells. In these fetal tissue cytokines IL-10 and IFN-γ were detected in CD4+ populations whereas in CD4- cells only IFN-γ was detected. Fetus is capable of generating an immune response own front to antigens transmitted by her mother, which induces the secretion of cytokines that act in synergy with the maternal antibodies confer a state of protection against infection and transmission of the parasite depends on factors specific to each mother, which may modify its ability to control such transmission to placental or systemic level.

694

SEVEN YEARS OF DATA COLLECTION AND ANALYSIS FOR THE LEISHMANIA DIAGNOSTIC LABORATORY AT WALTER REED ARMY INSTITUTE OF RESEARCH

Juan Mendez, Ioana E. Brasov, Li Y. Diao, John Tally, Max Grogl, Peter J. Weina

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

Leishmaniasis is a disease complex caused by 42+ species of protozoan parasites belonging to the genus *Leishmania*. Successful culturing of *Leishmania* parasites requires several techniques to insure proper identification of the species involved in the disease manifestation. An accurate and verifiable final diagnosis is the most important tool in finding the correct treatment for the affected individuals. Finding the proper diagnosis requires a series of laboratory techniques such as culturing and expanding the parasites and later identifying the species of *Leishmania* involved via molecular techniques. Our diagnostic laboratory is the only known certified laboratory by the *College of American Pathologist Laboratory,* approved by the *Clinical Laboratory Improvement Program* for high complexity testing of *Leishmania* disease, under the Department of Defense (DoD). This laboratory has seen a significant number of cases over the last 7 years with our involvement in the Middle East and elsewhere that *Leishmania* is endemic. Understanding that it is very important to discover, develop, implement and validate all possible methodologies to expand the knowledge in dealing with this neglected disease, we have reviewed seven years of data collected from submissions to this laboratory not only from DoD but from civilian sources as well. This data tabulation and interpretation provides to any interested party and most importantly to laboratory personnel who may encounter the need to assist in the diagnosis of the disease, a clear view of the past and present problems associated with diagnosing the disease, monitoring the incidence of exposure and infectivity in mapping different population groups, and discovering patterns of sample submission. This valuable information will hopefully help us to predict future problems and help us learn how to avoid them.

695

INFECTION AND PROLIFERATION DYNAMICS OF *TRYPANOSOMA CRUZI* IN HUMAN MACROPHAGES

Alexander Lankowski

Boston University School of Medicine, Boston, MA, United States

Significant geographic variation exists in the pathogenicity and virulence of different strains of *Trypanosoma cruzi*, the etiologic agent of Chagas disease. In particular, strains recently isolated from Bolivia are associated with a higher incidence of cardiac and gastrointestinal tract complications than previously characterized strains from Brazil and Peru. Macrophages play an important role in the immune response and pathogenesis of *T. cruzi* in humans. Not only are these cells important as innate mediators of parasite killing, but they are also capable of being infected by *T. cruzi* trypomastigotes and serving as vehicles for parasite proliferation. In order to compare the macrophage infection dynamics of different *T. cruzi* strains, an *in vitro* assay has been established which uses parasite strains induced to stably express β-galactosidase, allowing the measurement of relative parasite numbers via colorimetric reaction. Macrophages were isolated from 8 healthy volunteers and infected with β-galactosidase-expressing clones of either the strain "Bolivia" (strain DH29, clone L24) or "Tulahuen" (clone C4 - originally isolated from Brazil). Parasite numbers were then monitored by β-galactosidase activity for up to 72 hours. In 8/8 macrophage samples, the Tulahuen strain displayed both greater efficiency of initial infection and higher parasite density at all subsequent time points than did the Bolivia strain. These observations verify the usefulness of this assay as a method for comparing the virulence of different *T. cruzi* strains, and may also lend support to the hypothesis that the severity of Chagas disease pathology associated with certain strains may be more a result of an excessive immune response than of direct parasite-mediated toxicity.

696

EVALUATION OF THE ANTIBODY RESPONSE DIRECTED AGAINST TSETSE SALIVA ANTIGENS IN HUMANS

Emilie T. Dama

CIRDES, Bobo-Dioulasso, Burkina Faso

Saliva from blood sucking arthropods contains a rich array of pharmacologically active compounds whose primary function is to prevent the hemostatic mechanisms of the host. Furthermore, recent studies have shown that many of these saliva molecules are immunonogenic and elicit an antibody response. Such antibodies directed against saliva antigens may thus serve as marker of exposure to the bite by hematophagous vectors. The objective of this work was to assess if the IgG response directed against *Glossina* saliva was representative of the human-tsetse contact. For this purpose saliva was collected from *Glossina palpalis gambiensis* flies reared at CIRDES, and reactivity of human plasma was evaluated by an indirect ELISA. The study sample was composed of 301 plasma, from two active HAT foci in Guinea (Forécariah and Dubreka), two historical HAT foci in South-West Burkina Faso (Batié and Loropéni) and from volunteers living in Bobo-Dioulasso (a tsetse free area). The highest anti-saliva responses were observed in the HAT foci of Guinea, whereas responses were significantly lower in subjects living in Bobo-Dioulasso ($p<0.0001$) and in the Loropeni area ($p<0.0001$). High responses were also observed in Batié indicating that this population is still highly exposed to tsetse bites thus suggesting that the risk of re-emergence of HAT is important in this area notably in the context of the return of repatriates or seasonal workers from endemic areas from Côte d'Ivoire. Furthermore significant associations were also observed between the anti-saliva response and activities favoring human contact with tsetse flies (watering at backwater; $p=0.005$). Finally follow up in time of study subjects in endemic area suggest that the anti-saliva response is a dynamic process. As a whole, our results suggest that evaluation of the anti-saliva response may be a good alternative or complementary epidemiological tool to classical entomological methods to target most exposed populations and to evaluate efficiency of tsetse elimination programs.

697

DEVELOPING A MOUSE MODEL TO DETERMINE THE EFFECT OF SAND FLY SALIVA ON THE VISCERALIZATION OF *LEISHMANIA CHAGASI*

Melody Schmid[1], Claudio Meneses[2], Dia-Eldin Elnaiem[2], Gregory Lanzaro[1]

[1]University of California, Davis, Davis, CA, United States, [2]Vector Molecular Biology Unit, Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

Visceral Leishmaniasis (VL) is a vector borne disease that affects some of the world's poorest populations. In the New World VL is caused by the parasite *Leishmania chagasi*, which is transmitted by the sand fly *Lutzomyia longipalpis*. Sand fly saliva is known to have anti-hemostatic effects and immunomodulatory activities, which likely account for its ability to enhance cutaneous infections in BALB/c mice. These observations led us to hypothesize that sand fly saliva promotes the visceralization of *L. chagasi*. Indeed, preliminary data obtained using hamster hosts showed that the saliva of different populations of *Lu. longipalpis* have different effects on the visceralization of *L. chagasi*. However, the hamster model is limited because of the unavailability of immunological reagents, and the current BALB/c mouse model for cutaneous infection is unsuitable because it clears the visceral infection. The objective of this study is to develop a natural mouse model for VL that can be used to determine the effect of sand fly saliva on the visceralization of *L. chagasi*. We infected eight strains of mice via intraperitoneal injections of *L. chagasi* obtained from hamsters. Three immunodeficient strains (NuNu, NIHIII, and SCID Beige) showed signs of visceral disease up to sixteen weeks following injection, with typical parasite loads of 103 parasites per 100 host cells in the liver and spleen. Parasites isolated from the mouse spleens were then injected into naïve mice intradermally with sand fly saliva. The presence of parasites in the spleens of these mice supports the optimization of these strains as mouse models of VL. If sand fly salivary components prove to play an important role in visceralization they could be promising targets for a transmission blocking vaccine that could be integrated with current control methods.

698

UNBALANCED RATIO OF 28S AND 18S RIBOSOMAL RNAS IN *LEISHMANIA* AMASTIGOTES RECOVERED FROM BALB/C MICE LESIONS

María del Carmen Orozco-Fernández, Patricia Reyes-Uribe, Renzo Perales, Jorge Arevalo

Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Perú

Ribosome synthesis requires the coordinate gene expression of both ribosomal proteins and rRNA to satisfy living organism demands. *Leishmania* is an intracellular parasite that infects mammalian macrophages, including human beings. Whenever an infected sandfly vector feed on the skin, flagellated promastigotes invade the host cells and differentiate into non flagellated amastigotes. Complex changes should take place during promastigotes to amastigote conversion. Relative proportions of 18S rRNA and the 28S rRNA levels were measured in *Leishmania amazonensis*, These rRNAs were obtained either form cultured promastigotes or intracellular amastigotes obtained from ear dermis lesions in Balb/c mice. RNA from parasites in the log growth phase (day 3) and the early stationary phase (day 5) were obtained and subjected to reverse transcription with *Leishmania* specific primers. The ratio between 18S rRNA and 28S rRNA relative concentrations were measured by quantitative Real Time PCR. In promastigotes both logarithmic and stationary phase, like it was expected, the ratio between large and small rRNA was almost 1 (1.08±0.02). Noteworthy, when similar procedure was followed for amastigotes rRNA obtained from parasites present in lesions, the 28S rRNA was 8 fold times more abundant respect 18S rRNA. This observation was consistently observed in three different experiments. Functional ribosomes imply equimolar concentrations of large and small ribosomal sub units. Our results indicate that this is true for the promatigote stage but it is not the case for the amastigote stage. In this case, only a minor proportion of ribosomal subunits will be assembled like whole ribosomes. This non coordinated synthesis between 18S and 28b subunits, should lead to alterations of protein synthesis machinery, possibly encompassing parasite adaptation mechanisms to survive within the intracellular host environment.

699

EXPERIMENTAL VISCERAL LEISHMANIASIS IN ALYMPHOPLASIA (*ALY/ALY*) MICE

Saruda Tiwananthagorn[1], Kazuya Iwabuchi[2], Manabu Ato[3], Tatsuya Sakurai[1], Yuzaburo Oku[1], Ken Katakura[1]

[1]Laboratory of Parasitology, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Hokkaido, Japan, [2]Division of Immunobiology, Research Section of Pathophysiology, Institute for Genetic Medicine, Hokkaido University, Hokkaido, Japan, [3]Department of Immunology, National Institute of Infectious Diseases, Tokyo, Japan

Visceral leishmaniasis, caused by *Leishmania donovani* or *L. infantum* (*chagasi*), is one of the neglected tropical diseases causing significant health problems in humans and dogs worldwide. Relapses are frequent in immunocompromised patients and dogs in which parasites persist in the body even after treatment. Experimental infection of mice with *L. donovani* results in the development of organ-specific immunity in liver and spleen. However, a role of lymph node in parasite persistence and immune response has not been fully understood. We employed alymphoplasia *(aly/aly)* mice that lack lymph nodes and Peyer's patches, and demonstrate structural abnormalities of spleen and thymus due to a point mutation of NF-κB inducing kinase (NIK) as a model in this study. Intravenous inoculation of *aly/aly* and *aly/+* (control) mice with 5 x 107 *L. donovani* promastigotes was conducted and parasite burdens, liver histology and cytokine/chemokine responses were analyzed. The parasite burden was less in *aly/aly* mice in the early phase (4 weeks post-infection; WPI). However, the parasites remained in the liver of *aly/aly* mice at 12-WPI, when the most of parasites were removed in the *aly/+* mice. Impairment of granuloma formation and retention of infected cells in the liver were also demonstrated. Accordingly, higher parasite DNA was detected in the spleen, bone marrow, and peripheral blood of *aly/aly* mice at 12-WPI. In addition, RT-PCR/qPCR analysis revealed that 2- to 6-folds lower mRNA levels of chemokines and cytokines, including IP-10, MCP-1, RANTES, IFN-γ, TNF-α, GM-CSF and iNOS, were detected in the liver of *aly/aly* mice compared with the control mice at 4-WPI. These cytokines seemed to be necessary for the development of hepatic granuloma to resolve infection. Interestingly, mRNA level of FoxP3 in the liver of *aly/aly* mice was higher than that of *aly/+* throughout the course of infection. Defects of NF-κB pathway and/or the lymph nodes may elucidate the rather paradoxical responses (resistant in the early and susceptible in the late phase) to *L. donovani* infection in *aly/aly* mice.

700

PROPHYLACTIC EFFICACY OF TCVAC2 AGAINST *TRYPANOSOMA CRUZI* IN MICE

Shivali Gupta, Nisha Jain Garg

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

Trypanosoma cruzi is the etiologic agent of Chagas disease that is a major health problem in Latin America and an emerging infectious disease in the US. Previously, we have screened *T. cruzi* sequence database by a computational/bioinformatics approach, and identified antigens that exhibited the characteristics of good vaccine candidates. In this study, we have tested the protective efficacy of a multi-component heterologous prime/boost vaccine (TcVac2) constituted of the selected candidates in

a murine model of *T. cruzi* infection. ELISA and Flow Cytometry were employed to measure humoral and cellular responses. H&E and Masson's trichrome staining were used to evaluate pathologic changes in vaccinated and/or challenged mice. C57BL/6 mice vaccinated with TcVac2 elicited a strong antigen-specific antibody response dominated by IgG2b/IgG1 isotypes and moderate T cell proliferation. Upon challenge infection, TcVac2-vaccinated mice expanded the IgG2b/IgG1 antibody response and elicited a strong CD8+ T cell response associated with type 1 cytokines (IFN-γ & TNF-α) that resulted in control of acute parasite burden. In chronic phase, antibody response persisted in vaccinated mice; however, splenic activation of CD8+ T cells and IFN-γ/TNF-α cytokines subsided, and IL-4/IL-10 cytokines became dominant. The tissue parasitism, inflammation, and associated cell necrosis in skeletal and heart muscles of TcVac2-vaccinated chronic mice was undetectable. In comparison, control mice elicited mixed type1/type 2 responses to *T. cruzi* infection that persisted during the chronic phase, and contributed to parasite persistence and immunopathology in chagasic hearts. We conclude that TcVac2 immunization of mice elicited a strong antibody response and balanced type 1/type 2 T cell responses that were efficacious in controlling the acute and chronic tissue parasite burden and chronic immunopathology in chagasic hearts. Demonstrative experiments with similar vaccine formulation in dogs are being conducted and will be presented.

701

PERSISTENCE OF PARASITES IN SCARS CAUSED BY PAST HISTORY OF *LEISHMANIA MAJOR* INFECTION

Rabiaa-Manel Sghaier, Fouad Benhnini, Amor Zaatour, Nabil Bel-Haj-Hamida, Hanène Attia, Ghada Mkannez, Aymen Bali, Fatma Z. Guerfali, Afif Ben-Salah, Koussay Dellagi, **Dhafer Laouini**

Institute Pasteur of Tunis, Tunis-Belvedere, Tunisia

A vaccine to prevent leishmaniasis has been a goal for nearly a century based on the knowledge that a cured infection protects the individual from re-infection. It is generally believed that after spontaneous or chemotherapy induced healing of leishmaniasis, sterile cure is never achieved and that few residual living parasites will remain sequestered within some host cells that offer them a safe shelter. This statement is supported by data from experimental leishmaniasis in mice of susceptible or resistant phenotype, in which, live parasites could be recovered from lesions even after healing, and in which disease reactivation can be obtained by immune manipulation even after apparent complete cure. Whether maintenance of a long-term immune effector memory in humans will also require persistence of live parasites is presently unknown but stresses the importance of addressing the issue in zoonotic cutaneous leishmaniasis (ZCL) in the perspective of vaccine development. Our aim was to address the issue of *Leishmania major* parasite persistence vs. sterile healing in ZCL by analyzing biopsies of scars from healed volunteers. Skin-punch scars' biopsies (n=49) have been obtained from volunteers who had a past history of ZCL and who gave their written consent. The specimens were taken under sterile conditions and local anesthesia using a sterile single use puncher. Each specimen was divided into three parts: (*i*) the first sample was processed for quantitative real time PCR, (*ii*) the second was cultured *in vitro* on enriched medium and (*iii*) the third was inoculated into the footpad of susceptible BALB/c mice. We will present herein the preliminary data obtained on the persistence of live parasites on these collected scars' biopsies.

702

GENE EXPRESSION PROFILING REVEALS CONTRASTED EFFECTS OF *LEISHMANIA SPP* ON HUMAN MACROPHAGE TRANSCRIPTOME AND IDENTIFIES PARASITE SPECIFIC SIGNATURES

Fatma Z. Guerfali[1], Dhafer Laouini[1], Lamia Guizani-Tabbane[1], Florence Ottones[2], Khadija Ben-Aîssa[1], Jacques Marti[2], Koussay Dellagi[1]

[1]Institut Pasteur de Tunis, Tunis, Tunisia, [2]Institute of Human Genetics, Montpellier, France

Zoonotic cutaneous leishmaniasis caused by Leishmania (L) major is presented primarily as localized self healing cutaneous sores with a broad range of clinical variations. In addition, L. infantum is responsible in the Mediterranean basin for visceral (VL) or sporadic cutaneous leishmaniasis (SCL). Macrophages are the main target of these parasites and their shelter; they participate to shape the host immune response in an attempt to ultimately kill the parasites. As such, they could be operationally used as sensors to screen for functional diversity between parasites. To identify inter and intra-species' differences, we performed a gene expression profiling in human macrophages infected with one of the four selected *Leishmania* parasite strains: two *L. major* strains expressing contrasted levels of virulence (high vs. low virulence) according to their experimental pathogenicity and two *L. infantum* strains expressing contrasted tropisms (visceral vs. cutaneous), using Serial Analysis of Gene Expression technology (SAGE). Using various analysis tools, we were able to discriminate between the human and parasite transcripts. A set of about two hundred human genes showed statistically significant differential expression of genes in macrophages infected with either high- or low-virulent *L. major* strains and viscerotopic or dermotropic *L. infantum* strains. These genes, belonging to different functional families, are likely to be involved in the control of parasite multiplication and may play a dominant role in determining the clinical expression of disease. Further studying of these selected genes may help better understanding the physiopathology of the disease and improving anti-Leishmania drugs' screening.

703

COMPARISON OF GENE EXPRESSION PATTERNS AMONG *LEISHMANIA BRAZILIENSIS* CLINICAL ISOLATES DIFFERING IN SUSCEPTIBILITY TO PENTAVALENT ANTIMONY

Vanessa K. Adaui[1], Kathy Schnorbusch[2], Mirko Zimic[3], Andrés Gutiérrez[3], Saskia Decuypere[2], Manu Vanaerschot[2], Simonne De Doncker[2], Ilse Maes[2], Alejandro Llanos-Cuentas[1], François Chappuis[4], Jorge Arévalo[1], Jean-Claude Dujardin[2]

[1]Institute of Tropical Medicine Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Institute of Tropical Medicine Antwerp, Antwerp, Belgium, [3]Laboratorios de Investigación y Desarrollo, Universidad Peruana Cayetano Heredia, Lima, Peru, [4]Hôpitaux Universitaires de Genève, Geneva, Switzerland

The clinical value of the antileishmanial treatment pentavalent antimonials (SbV) is threatened by the emergence of drug resistance. The evaluation of drug susceptibility of *Leishmania* parasites still depends on *in vitro* biological assays, which are labour-intensive and time-consuming. Molecular markers are urgently needed to simplify the monitoring of SbV-resistance. This paper evaluates the potential of gene expression profiling to characterize *L. braziliensis* clinical isolates differing in SbV susceptibility. Twenty-one isolates were analyzed during *in vitro* promastigote growth for differential expression of 13 genes involved in SbV metabolism, oxidative stress or housekeeping functions. Our study revealed homogeneous expression profiles for most examined genes among the phenotypically different isolates. Two genes, *ODC* (encoding ornithine decarboxylase) and *TRYR* (encoding trypanothione reductase), showed a significantly higher expression rate in the group of SbV-resistant compared to the group

of SbV-sensitive parasites (P<0.01). However, both markers have a low sensitivity, and thus only explain a small part of the drug resistance within present sample. Our results might be explained by (i) the occurrence of a pleomorphic molecular mechanism leading to SbV resistance and/or (ii) the definition of the *in vitro* SbV-susceptibility phenotypes here compared. Further exploration should also consider analysis of the amastigote stages.

705

MICROENCAPSULATION OF TRANSGENIC *BACILLUS SUBTILIS* WITHIN CHITOSAN-COATED ALGINATE MICROSPHERES

Adam Forshaw

University of New Mexico School of Medicine, Albuquerque, NM, United States

Leishmaniasis is a global health concern with an estimated 12 million people infected and 367 million at risk. Visceral leishmaniasis (VL) is the most devastating form of the disease with mortality approaching 100% if left untreated. This disease is caused by *Leishmania donovani*, a protozoan kinetoplastid flagellate, and is transmitted predominantly by the sandfly, *Phlebotomus argentipes*. We have previously identified *Bacillus subtilis* as a commensal microbe within the gut of *P. argentipes*, and are developing this microbe for paratransgenic control of *L. donovani* transmission. By supplementing sand fly larvae with modified B. subtilis, we have demonstrated the transstadial delivery of this microbe to the emergent sand fly. Field application of the paratransgenic strategy for control of VL would require measures that would minimize gene spread into the environment. This work introduces the concept of second generation paratransgenics in which advanced material engineering at the micro-scale level is used for targeted release of the modified microbes to specific sites of pathogen residence within the arthropod itself. To this end, we have encapsulated *B. subtilis* within a chitosan-coated alginate (CCA) micro-particle using a modified aerosolization-coacervation process. We have demonstrated regulated release of *B. subtilis* from these particles specifically at neutral pH, and will show the stability of the CCA particles under a variety of soil conditions. We plan to transstadially deliver these muco-adhesive, pH-gated CCA particles to *P. argentipes*, and expect the particles to only release their microbial payload following the first blood meal and therefore biological pH change (from acidic to neutral) in the sand fly gut. This novel approach for delivery of modified microbes for paratransgenic control should alleviate concerns relating to containment of modified microbes, issues related to environmental spread of recombinant bacteria and potential horizontal gene transfer of foreign DNA to environmental microbiota.

706

IN VITRO EVALUATION OF THE EXPRESSION OF DYSTROPHIN IN CARDIOMYOCYTES STIMULATED WITH SERUM OF MICE EXPERIMENTALLY-INFECTED WITH *TRYPANOSOMA CRUZI*

Lygia M. Malvestio, Cibele M. Prado, Erica C. Campos, Mara Rubia Celes, Valdecir Blefari, João Santana da Silva, Marcos Antonio Rossi

University of Sao Paulo, Ribeirão Preto, Brazil

Dystrophin is implicated in the maintenance of the cell shape, mechanical resistance and signal transduction to cardiomyocytes. Results from our laboratory have been showing decreased expression of the dystrophin glycoprotein complex (DGC), especially dystrophin, in experimentally-induced *Trypanosoma cruzi* myocarditis, both in the acute and chronic phase of the disease. This study tests the hypothesis that serum of mice experimentally-infected with *T. cruzi* affects the expression of dystrophin in cultured newborn cardiomyocytes. Cultured newborn cardiomyocytes were stimulated 5 days after their first spontaneous beating with serum of mice infected with *T. cruzi* for 24 hours. Serum was obtained from male C57Bl/6 mice infected with *T. cruzi* in the peak (12 days post infection) of cardiac tissue inflammation. Imunofluorescence (IF) staining and Western blotting (WB) were performed for evaluation of the expression of dystrophin, phalloidin, troponin, TNF-α, calpain, iNOS, and NF-κB. The IF for phalloidin and troponin confirmed the presence in cardiomyocytes. The immunostaining for dystrophin in control cells was localized around the nucleus and subsarcolemal regions. The cardiomyocytes stimulated with serum of *T. cruzi* infected mice showed decreased expression of dystrophin. This decrease was confirmed by WB analysis. The expression of TNF-α, calpain, iNOS, and NF- κB was increased in the cells. In conclusion, our results lend support to the hypothesis that serum of *T. cruzi* infected mice directly affects expression dystrophin. It can be hypothesized that TNF-α and iNOS could activate NF-κB and contribute to dystrophin disruption damage through activation of intracellular proteases, such as calpain in the present study.

707

EARLY DYSTROPHIN DISRUPTION IN THE PATHOGENESIS OF EXPERIMENTAL CHRONIC CHAGAS CARDIOMYOPATHY

Cibele M. Prado[1], Mara Rubia Nunes Celes[1], Lygia M. Malvestio[1], Erica C. Campos[1], Linda A. Jelicks[2], Herbert T. Tanowitz[2], Marcos A. Rossi[1]

[1]Faculty of Medicine of Ribeirao Preto, Ribeirao Preto, Brazil, [2]Albert Einstein College of Medicine, New York, NY, United States

The most intriguing aspect of chronic Chagas cardiomyopathy (CCC) is that it takes a long time to develop after the initial infection by the protozoan *Trypanosoma cruzi*. Chagas disease is characterized by three phases: acute, latent, and chronic, the heart as the most severely and frequently involved organ. Similarly to CCC, cardiac complications due to cardiomyopathy appear later in life in Duchenne muscular dystrophy due to an absence of or defect in dystrophin. In this study we tested the hypothesis that dystrophin expression could be decreased in the beginning of *T. cruzi*-infected mice preceding the late development of cardiomyopathy. Male CD1 mice were infected with 5×104 trypomastigotes of the Brazil strain of *T. cruzi*. Mice were killed 30 and 100 days post infection (dpi) and the intensity of inflammation, fibrosis and dystrophin expression were evaluated. Echocardiography, magnetic resonance and positron emission tomography were evaluated from days 15-100pi. At 30dpi there was an intense and diffuse lymphomononuclear myocarditis, disruption of myofibers, and multiple intracellular parasite nests. The inflammation subsided significantly and parasites were not detected at 100dpi. Dystrophin immunolabeling was focally reduced or completely lost in cardiac myocytes at 30dpi, this reduction maintained up to 100dpi. Ejection fraction was significantly reduced at 60-100dpi. The RV was markedly dilated from 30-100dpi and the LV wall thickness was increased at 100dpi. Infected mice displayed greater uptake of glucose from days 15-100pi. In conclusion, a late cardiomyopathy developed in mice chronically infected with *T. cruzi* could be associated with dystrophin loss.

708

POPULATION PHARMACOKINETICS (PK) OF PYRONARIDINE IN ADULT PATIENTS WITH UNCOMPLICATED ACUTE *PLASMODIUM FALCIPARUM* OR *PLASMODIUM VIVAX* MALARIA

Thitima Wattanavijitkul[1], Stephan Duparc[2], Isabelle Borghini-Fuhrer[2], Chang-Sik Shin[3], Lawrence Fleckenstein[1]

[1]College of Pharmacy, The University of Iowa, Iowa City, IA, United States, [2]Medicines for Malaria Venture, Geneva, Switzerland, [3]Shin Poong Pharmaceuticals, Seoul, Republic of Korea

A novel pyronaridine/artesunate (PA) combination is being developed for the treatment of malaria. The purpose of this study is to determine population PK of pyronaridine (PYR) in adult patients from Africa and South East Asia participating in Phase II and Phase III clinical trials who received treatment with PA once-a-day for 3 days. A total of 699 blood concentrations collected from 321 adult patients, aged 15-60 years, with uncomplicated *falciparum* and vivax malaria were included in

this analysis. Blood PYR concentrations were natural log-transformed. Two- and three-compartment models were fitted to the data using NONMEM. The influence of covariates (age, sex, weight, height, body mass index, lean body weight (LBW), red blood cell indices, parasite count, liver function tests and geographic regions) on PK parameters was tested. Bootstrap analysis and visual predictive check (VPC) were done to evaluate the model. A two-compartment model with first order absorption and elimination best described the data. Inter-subject variability (ISV) of absorption rate constant (Ka), oral clearance (CL/F), and apparent central compartment volume (V2/F) were described using an exponential error model. The ISV of peripheral compartment volume (V3/F) and intercompartmental clearance (Q/F) could not be estimated. A log error model best described residual variability. Only LBW was found to be a significant predictor of V2/F. Typical model parameter estimates (%ISV) were Ka 29.3 1/d (109%), CL/F 1180 L/d (50%), V2/F 8540 L (82%), V3/F 13200 L and Q 1720 L/d. The estimated elimination half-life was 18 days. The final model provided estimates within the 95% confidence intervals obtained by 1000 bootstrap runs. VPC showed that the final model adequately captured the majority of the data. In conclusion, a 2-compartment model was well-fitted to pyronaridine data. LBW was an important covariate of V2/F in adult patients. The parameter estimates were plausible. The final model was robust and sufficiently captured the overall PYR PK.

709

PUNICALIN AND PUNICALAGIN FAILS CEREBRAL MALARIA?

Deepak X. Bhattacharya

Oddisi Research Laboratory, Bhubaneswar, Orissa, India

In *AJTMH* 2003, 2004 & Multi Lateral Initiative on Malaria Yaounde-05, introduced Indo medicinal fruit called *Dalimba* (P granatum) having therapeutic & prophylactic efficacy against drug resistant malaria *Pf -Pv*, alongwith active moieties, efficacy spectrum, scavenger/anti-oxidative/anti-inflammatory, anti-viral, adjuvant, & K+ as driver candidate (as reported previously). Is a CAM, a invention on global basis termed OMARIA-Orissa Malaria Research Indigenous Attempt (BBC & Eco.Times, India, 24/25-10-2000). *In vitro* results presented from authentic source (as reported previously). Decadal mono-station continuous large scale use (Koraput-Orissa-India) indicates that >15000 individuals & whole families who all had consumed OMARIA seem to block transmission for years end, no signs of resistance, non ever developed cerebral malaria. Why? OMARIA contains 1 small, stable, (A-i) Ellagic acid rich in H+, clinically indicates hepatogen, pylori & nephron toxic symptoms at sustained/bolus doses, & 2 unstable, large, hydrolytic Ellagitanins (B-ii) Punicalagin & (B-iii) Punicalin/folin of low H+, rich in OH, wholly non toxic (C-i) K+. Which Group inflicts thus ? Or A + B + C = synergistic action ? In Indian natural sources, group B is > group-A, non confounding. K+ binds exclusively to group B, which has longer bio-availability (Sreeram-04; Soh-08). Homeopaths use diluted Acetic acid; Ellagic Acid (0.25~5%) as internal medicine to treat tertian malaria *Pf-Pv*. Initially all case report relief, then rebound with hepatic, digestive, bowl problems, long use complication = drug failure. Very same cases report feel good factor & eventual 'clinical clear status' with OMARIA. Tertian cases treated with fruits & herbs rich in group A (Chestnut bark) & non from group B is ineffective & also non gametocidal. Group A do not deliver prophylaxis nor as safe. Drug dose therapeutic response of Group-A is even not equal to Artimisinin. Group B-ii & B-iii → slow onset, long acting, potent, therapeutic, prophylactic, pregnancy safe & Gametocidal even at sub-clinical doses. K+ (C-i) thwarts neuro-cerebral morbidity.

710

ACTIVITY OF 8-AMINOQUINOLINE (8AQ) ANTIMALARIAL DRUG CANDIDATES AGAINST BLOOD STAGE *PLASMODIUM FALCIPARUM*

Yarrow Rothstein[1], Jacob Johnson[1], Aruna Sampath[1], William Ellis[1], Dhammika Nanayakkara[2], Ikhlas Khan[2], Larry Walker[2], Alan Magill[1], Colin K. Ohrt[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS, United States

8-aminoquinolines (8AQ) may prove critical for malaria elimination efforts since they target hypnozoites and *Plasmodium falciparum* (Pf) gametocytes. It is unclear if 8AQs could have a role in targeting the remaining component of the transmission reservoir - asymptomatic blood stage parasitemia. The key toxicity in this class will soon be addressed with animal models predicting hemolytic toxicity in G6PD-deficiency. The Walter Reed Army Institute of Research chemical information system contains data on 1803 8AQs. Of these, 106 have been tested *in vitro* against Pf, with 33 having IC50's < 200 ng/ml. Of 1457 compounds assessed in a single dose mouse *P. bergheii* blood stage model, 195 had curative activity. Ten out of ten assessed as single agents in a 3-day Aotus monkey *P. falciparum* treatment protocol had curative activity. We plan to evaluate the *in vitro* efficacy of all available 8AQs against *P. falciparum in vitro*, to confirm their lack of cross-resistance with standard antimalarial drugs, and to determine if efficacy can be separated from hemolytic toxicity. The existing data and new data relevant to *P. falciparum* blood stage efficacy and therapeutic index will be presented.

711

PHARMACOKINETICS (PK) AND QTC CHANGES AFTER COADMINISTRATION OF TAFENOQUINE (TQ) AND CHLOROQUINE (CQ) IN HEALTHY VOLUNTEERS

Ann K. Miller[1], Khadeeja Mohamed[2], Li Ye[3], Emma Harrell[4], Sharon Baptiste-Brown[1], Jörg-Peter Kleim[2], Colin Ohrt[5], Stephan Duparc[6], Andrew Beelen[7], Alison Webster[2], Sandy Griffith[8]

[1]GlaxoSmithKline Pharmaceuticals, King of Prussia, PA, United States, [2]GlaxoSmithKline Pharmaceuticals, Uxbridge, Middlesex, United Kingdom, [3] GlaxoSmithKline Pharmaceuticals, Collegeville, PA, United States, [4]GlaxoSmithKline Pharmaceuticals, Harlow, Essex, United Kingdom, [5]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [6]Medicines for Malaria Venture, Geneva, Switzerland, [7]Miriad Pharmaceuticals, Salt Lake City, UT, United States, [8]GlaxoSmithKline Pharmaceuticals, Research Triangle Park, NC, United States

Tafenoquine (TQ) is an 8-aminoquinoline in development for the treatment and radical cure of *Plasmodium vivax* malaria. TQ will be coadministered (not coformulated) with chloroquine (CQ). The PK and safety of TQ, CQ and its desethyl metabolite (DECQ) were evaluated when given concomitantly compared to TQ or CQ given alone in healthy adults. Due to the long half-lives of both TQ and CQ, a double-blind, parallel group design with 20 subjects/group was used: CQ alone (600mg on D1 and D2+300mg on D3); TQ alone (450mg on D2 and D3); and CQ plus TQ at the same doses and times. Frequent blood samples for PK were taken on D2 and D3 with additional samples taken out to D56. 12-Lead ECGs were collected in triplicate on D-1; pre-dose, 2 and 12 h post dose on D1-3; and daily on D4-7. Plasma TQ, CQ and DECQ concentrations were determined using an HPLC-MS/MS method. PK parameters were determined using non-compartmental methods. Only a short term significant effect on TQ PK was seen on D2 when taken with CQ (Cmax and AUC(0-24) increased 38% and 24%, respectively) with no significant effects seen for Cmax and AUC(0-24) on D3, AUC(0-∞) and t1/2. TQ had no significant effect on CQ and DECQ PK. No subjects had a QTcF >480msec or a change from baseline ≥ 60msec. QTcF intervals increased when treated with CQ alone but there was no trend for increased QTcF intervals in those treated