and the IEC program from MCs were significantly higher after the intervention and were significantly higher among the intervention group than that of the control group. Success of malaria control requires not merely the investigation and promptly treatment, proper health education and promotion about treatment are also necessary. IEC programs are a powerful and effective means for encouraging the vivax malaria patients to adhere to their treatments.

168

EFFICACY OF ARTEMETHER-LUMEFANTRINE POWDER FOR SUSPENSION (CO-ARTESIANE®) COMPARED WITH ARTEMETHER-LUMEFANTRINE (COARTEM®) TABLETS IN THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN CHILDREN UNDER FIVE YEARS IN WESTERN KENYA: A RANDOMIZED OPEN-LABEL TRIAL

Elizabeth A. Juma

Kenya Medical Research Institute, Kisumu, Kenya

Several malaria control programs have adopted artemether/lumefantrine as the treatment of choice for uncomplicated malaria. Six-dose artemether/lumefantrine tablets are highly effective and safe for the treatment of infants and children weighing between 5 and 25 kg with uncomplicated *P. falciparum* malaria. However, oral paediatric formulations are urgently needed, as the tablet is unsuitable for young children who cannot swallow whole tablets and often expel the drug due to the bitter taste of the crushed tablets. We compared PCR corrected cure-rates on day 28 in children aged 6 - 59 months treated with either artemether/lumefantrine tablets (Coartem®) or artemether/lumefantrine suspension (Coartesiane®) for uncomplicated falciparum malaria in Western Kenya. The study design was an open-label randomized single centre trial. 247 children aged 6 - 59 months, weighing ≥5 kg with uncomplicated falciparum malaria were randomized to receive either 6-dose AL tablets or 3-dose AL suspension over 3 days of treatment and followed up for a total of 28 days. Respectively, 125/134 (93%) and 122/133 (94%) of children in the AL tablets and AL suspension arms completed followed up. A per protocol analysis revealed a PCR-corrected parasitological cure rate of 95.2% at Day 28 in the AL tablets group and 92.6% in the AL suspension group. Both drugs effectively cleared gametocytes and were well tolerated, with no difference in the overall incidence of adverse events. The 3- once daily dose of artemether-lumefantrine powder for suspension (Co-artesiane) was as efficacious as the 6-dose artemether-lumefantrine tablet (Coartem®) for the treatment of uncomplicated malaria in children below 5 years of age in western Kenya.

169

ARE MALARIA TREATMENT EXPENDITURES CATASTROPHIC TO DIFFERENT SOCIO-ECONOMIC AND GEOGRAPHIC GROUPS AND HOW DO THEY PAY IN SOUTHEAST NIGERIA?

Obinna Onwujekwe[1], Kara Hanson[2], Benjamin Uzochukwu[3]

[1]London School of Hygiene and Tropical Medicine and University of Nigeria, Enugu, Nigeria, [2]London School of Hygiene and Tropical Medicine, London, United Kingdom, [3]College of Medicine, University of Nigeria, Enugu, Nigeria

The objective of this study was to determine the incidence and level of inequities in catastrophic health spending due to malaria treatment expenditures as well as socio-economic and geographic differentials in payment strategies for treatment of malaria in southeast Nigeria. The study was undertaken in three urban and three rural towns in southeast Nigeria using pre-tested structured questionnaires to collect information on from a random sample of 2250 householders. Catastrophic malaria treatment expenditure was computed as the percentage of average monthly malaria treatment expenditure in average monthly non-subsistence household expenditure (minus food expenditure). A socio-economic status (SES) index and comparison of rural-urban differences was used to examine inequity. The average treatment cost for malaria was 796.5 Naira for adults and 789.0 Naira for children. Malaria treatment expenditure as a proportion of monthly household non-food expenditure was 7.8%, 8.5%, 5.5% and 3.9% for the most poor, very poor, poor and least poor SES groups respectively. The percentages were 7.1% and 5.0% for rural and urban dwellers respectively. Out-of-pocket payment (OOP) was used by more than 95% of the people to pay for malaria and there were no SES and rural-urban differences in its use. There was almost a total absence of insurance payment mechanisms and fee exemptions. In conclusion, there were socio-economic and geographic inequities in the expenditures and payment catastrophe due to malaria treatment. The treatment expenditure depleted more of the income of the two worse-off SES (Q1 and Q2) and of the rural dwellers. In the presence of the high incidence of poverty in Nigeria and with more than 50% of the people living below the poverty lines, the health expenditures to poor SES groups are catastrophic using a threshold of 5%. Abolition of user fees, move from OOP to pre-payment mechanisms, improving physical access to appropriate malaria treatment services, subsidies and deferrals should be instituted by government and donors so as to engender financial risk protection from malaria treatment.

170

A SINGLE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRIC METHOD FOR THE SIMULTANEOUS DETERMINATION OF 14 ANTIMALARIALS AND METABOLITES IN HUMAN PLASMA

Eva-Maria Hodel[1], Boris Zanolari[2], Thomas Mercier[2], Jennifer Keiser[1], Thierry Buclin[2], Jérôme Biollaz[2], Blaise Genton[1], Laurent A. Decosterd[2]

[1]Swiss Tropical Institute, Basel, Switzerland, [2]Division of Clinical Pharmacology, University Hospital, Lausanne, Switzerland

Among the various determinants of treatment response, achieving a sufficient blood concentration is pivotal to curing malaria. For an ongoing multicenter study assessing the influence of pharmacogenetics on treatment efficacy, we developed a liquid chromatography-tandem mass spectrometric method (LC-MS/MS) enabling in 200 µl of plasma the simultaneous determination of 14 antimalarial drugs and metabolites (artemether, artesunate, dihydroartemisinine, lumefantrine, desbutyl-lumefantrine, amodiaquine, desethyl-amodiaquine, piperaquine, pyronaridine, mefloquine, chloroquine, quinine, pyrimethamine and sulfadoxine). Plasma is purified by a combination of protein precipitation, evaporation and reconstitution in Methanol/ammonium acetate 20 mM (pH=4.0) 50:50. Reverse-phase chromatographic separation of antimalarial drugs is performed using a gradient elution of 20 mM ammonium acetate and acetonitrile containing both 0.5% formic acid, followed by intensive rinsing and re-equilibration to the initial solvent composition up to 21 min. Analyte quantification, using matrix-matched calibration samples is performed by electro-spray ionisation-triple quadripole mass spectrometry by selected reaction monitoring detection using the positive mode. The method has been validated according to the FDA recommendations, including assessment of extraction yield, matrix effect variability as well as antimalarials short- and long-term stability in whole blood and plasma. The method is precise (inter-day CV% :4.0-12.2 %) and sensitive (lower limits of quantification : 0.15 to 3.0 ng/mL and 1.5 to 5 ng/mL for basic/neutral antimalarials and artemisinine derivatives, respectively). This sensitive and accurate analytical method, using small sample material, allows the simultaneous assessment of the concentration of several drugs, which is highly relevant at the era of drug combinations. It could be conveniently applied within the frame of the World AntiMalarial Resistance Network (WARN) aimed at ascertaining whether resistance to treatment is caused by intrinsic parasite resistance or low drug levels. Our method detecting most of the antimalarials currently used makes it also possible to determine whether patients have taken treatments prior to the medical visit. Further developments of the LC-MS/MS assay in whole blood and red blood cells -at the site of pharmacological action- are currently underway.

171

EXPOSURE TO LUMEFANTRINE IN INFANTS AND CHILDREN RECEIVING ARTEMETHER-LUMEFANTRINE FOR UNCOMPLICATED MALARIA: IMPACT OF AFRICAN DIET COMPONENTS

Steffen Borrmann[1], William M. Sallas[2], Anne-Claire Marrast[3], Gilbert Lefèvre[4], Steven E. Kern[5]

[1]Kenya Medical Research Institute/Wellcome Trust Collaborative Centre, Kilifi District Hospital, Kilifi, Kenya, [2]Department of Modeling and Simulation, Novartis Pharmaceuticals Corporation, East Hanover, NJ, United States, [3]Department of Tropical and Infectious Diseases, Novartis Pharma AG, Basel, Switzerland, [4]Department of Drug Metabolism and Pharmacokinetics, Novartis Pharma AG, Basel, Switzerland, [5]Department of Pharmaceutics, University of Utah, Salt Lake City, UT, United States

Artemether-lumefantrine (AL) shows high 28-day cure rates (~98%) when used to treat children with uncomplicated malaria caused by *Plasmodium falciparum*. The absorption of lumefantrine is enhanced when AL is taken with food. We evaluated the effect of food on lumefantrine exposure in children with uncomplicated *P. falciparum* malaria. Data were obtained from a randomized, multicenter study of two AL formulations (commercially-available crushed tablet versus dispersible). Co-administration of food with AL doses was recommended in the study protocol. The plasma concentration of lumefantrine was measured in patients randomized to receive the crushed tablet, and used to construct a two-compartment pharmacokinetic model for lumefantrine. This model was used to estimate relative lumefantrine exposure (i.e. relative dose absorbed) in patients who consumed different foodstuffs at the time of AL administration versus those who ate nothing. In total, 315 patients received 1,886 AL doses. The most frequent meals consumed at the time of AL administration were milk alone (57.0%), pancakes alone (27.7%), porridge alone (3.9%), other (1.2%) or none (9.9%). There was a 1.72 (95% CI: 1.39-2.13) relative increase in mean lumefantrine absorption in patients consuming milk and a 2.62 (95% CI: 2.09-3.28) relative increase in those eating pancakes compared to patients who ate no meal. Of these 315 patients, 300 were included in the primary efficacy analysis: 298/300 experienced parasitological cure (PCR-corrected) at Day 28. There were 20 subjects who consumed no food at the time of any AL dose, and all 20 of these individuals experienced parasitological cure at Day 28. In conclusion, consumption of typical African meals increases lumefantrine absorption in children receiving AL for uncomplicated malaria. In this study, the efficacy of AL was high and was not affected by variation in meals consumed at the time of AL administration.

172

TYPICAL AFRICAN DIETS: FAT CONTENT AND ABSORPTION OF ARTEMETHER-LUMEFANTRINE

Zulfiqarali C. Premji[1], Salim Abdulla[2], Bernhards Ogutu[3], Alice Ojwang-Ndong[4], Catherine Falade[5], Issaka Sagara[6], Cecilia A. Ankrah[7], Nathan A. Mulure[8], Obiyo Nwaiwu[9], Gilbert Kokwaro[10]

[1]Muhimbili University of Health and Allied Sciences, Dar-es-Salaam, United Republic of Tanzania, [2]Ifakara Health Research and Development Centre, Dar-es-Salaam, United Republic of Tanzania, [3]Centre for Clinical Research, Kenya Medical Research Institute, Kisumu, Kenya, [4]Centre for Nutrition Education and Research, Nairobi, Kenya, [5]Department of Clinical Pharmacology, University College Hospital, Ibadan, Nigeria, [6]Malaria Research and Training Center, University of Bamako, Bamako, Mali, [7]Ministry of Health and Korle Bu Teaching Hospital, Accra, Ghana, [8]Novartis Pharma AG, Nairobi, Kenya, [9]Novartis Pharma AG, Lagos, Nigeria, [10]Kenya Medical Research Institute (KEMRI)/Wellcome Trust Research Programme and College of Health Sciences, University of Nairobi, Nairobi, Kenya

Artemether-lumefantrine (AL) is recommended for treatment of acute, uncomplicated malaria caused by *P. falciparum* in endemic and multi-drug resistance areas, and is both highly effective and well-tolerated. Absorption of lumefantrine, a lipophilic compound, is variable following oral administration. Oral AL given concomitantly with food or drink that contains fat shows improved absorption, due to the enhanced solubility of lumefantrine in fat. It has been determined in healthy volunteers that concomitant administration of AL with 1.28g fat (given as 40mL soya milk) increases lumefantrine absorption (AUC) five-fold. This small amount of fat ensured delivery of 90% of the lumefantrine exposure obtained with 16g of fat given as 500mL soya milk. We assessed the fat content of some common foods from seven African countries, and average total fat intake per day in these countries, based on published data. Most foodstuffs were found to contain some fat. The fat content of maize, a staple food common to these countries, is 4.8g fat/100g dry weight. The intake of fat is supplemented by widespread use of plant oils in food preparation (e.g. red palm, groundnut, coconut and sesame oils). The average total fat intake per person during 2001-2003, for example, was 10, 32, 31, 59, 49, 63 and 46g/day in Burundi, Uganda, Tanzania, Cote d'Ivoire, Kenya, Nigeria and Mali, respectively. Breast milk from African women has a fat content of approximately 35g/L, and a typical breast milk feed contains >2g fat. In conclusion, the fat content of common African diets or breast milk should be adequate to ensure optimal lumefantrine absorption from AL.

173

COMMUNITY-LEVEL DEPLOYMENT OF ARTEMETHER LUMEFANTRINE (AL) WITH RAPID DIAGNOSTIC TESTING: EFFECT ON MALARIA OUTCOMES AND RESOURCE UTILIZATION IN A RURAL SETTING

Hailemariam Lemma[1], Alem Desta[1], Edward Fottrell[2], Gebre Ab Barnabas[1], Angela Bianchi[3], Andrea Bosman[4], Peter Byass[4], Gianfranco Costanzo[5], Aldo Morrone[6], Nathan Mulure[7], Luigi Toma[8]

[1]Tigray Health Bureau, Mekelle, Ethiopia, [2]Department of Health and Clinical Medicine, Umea University, Umea, Sweden, [3]Novartis Pharma, Origgio, Italy, [4]WHO Global Malaria Programme, Geneva, Switzerland, [5]Italian Ministry of Health, Rome, Italy, [6]National Institute for Health Migration and Poverty, Rome, Italy, [7]Novartis Pharma, Nairobi, Kenya, [8]S. Gallicano Hospital, Rome, Italy

Prompt diagnosis of malaria and administration of artemether lumefantrine (AL) at community level may improve malaria control. A two-year observational study was undertaken during May 2005 to June 2007 to evaluate the impact and potential cost savings of deploying AL with rapid diagnostic testing through community health workers (CHWs). The study was undertaken in the Alamata and Raya Azebo districts of Tigray, Northern Ethiopia. AL was first-line antimalarial treatment in both districts. In the intervention district (Alamata) AL was provided at village level through 33 trained CHWs as well as in state health facilities, with phased introduction of rapid diagnostic tests (RDTs) by 50% of the CHWs during year 2 of the project. In the control district, AL was provided only through general health services without involvement of CHWs. In the intervention district, 75,654 patients with suspected malaria were managed by CHWs and 54,774 at health facilities. During the malaria epidemic May-October 2005, there was a significantly lower risk of malaria-specific death in the intervention district vs the control district (adjusted incidence rate ratio 0.52, 95% CI 0.31-0.87, P=0.014). Among 5,122 patients assessed by CHWs using RDTs, only 527 (10.3%) were *Plasmodium falciparum* positive and received AL. Patients testing RDT negative were treated with chloroquine, at a net cost saving of US$1.41 per patient (based on AL public sector price during 2001-2006). In contrast, 38% patients examined with microscopy or RDTs at health post and clinics were diagnosed with *P. falciparum* infection and received AL. Community satisfaction with the project was high due to improved treatment access and reduced waiting time at health clinics. In conclusion, in this large-scale study in a resource-constrained rural setting, community-level rapid diagnosis of malaria and AL treatment was associated with a significantly lower risk of malaria-specific mortality. Use of RDTs facilitated targeted AL treatment and generated significant cost savings.

174

A QUALITATIVE STUDY OF THE FEASIBILITY AND COMMUNITY PERCEPTION ON THE EFFECTIVENESS OF ARTEMETHER-LUMEFANTRINE USE IN THE CONTEXT OF HOME MANAGEMENT OF MALARIA IN SOUTHWEST NIGERIA

Ikeoluwapo O. Ajayi[1], Catherine O. Falade[1], Benjamin O. Olley[2], Bidemi Yusuf[1], Sola Gbotosho[1], Toyin Iyiola[3], Omobola Olaniyan[3], Christian Happi[1], Kaendi Munguti[4], Franco Pagnoni[5]

[1]College of Medicine, University of Ibadan, Ibadan, Nigeria, [2]University of Ibadan, Ibadan, Nigeria, [3]Ministry of Health, Oyo State, Ibadan, Ibadan, Nigeria, [4]University of Nairobi, Nairobi, Kenya, [5]World Health Organisation, Geneva, Switzerland

In Nigeria ACT use at the community level has not been evaluated and the use of antimalarial drugs (commonly chloroquine (CQ)) at home has been shown to be largely incorrect. The treatment regimen of ACT is however more complicated than that of CQ. There is thus a need to determine the feasibility of using ACT at the home level and determine community perception on its use. A before and after qualitative study using key informant interviews (KII) and focus group discussions (FGDs) was conducted in selected villages in Ona-Ara local government area. At baseline, 14 FGDs and 14 KIIs were conducted. Thereafter, community medicine distributors (CMDs) were trained in each village to dispense artemeter-lumenfantrine (AL) to febrile children aged 6-59 months presumed to have uncomplicated malaria. After one year of drug distribution, nine KIIs and 10 FGDs were conducted. Participants and key informants were mothers and fathers with children under five years, traditional heads of communities, opinion leaders and health workers. None of the participants have heard of AL prior to study. Participants were favourably disposed to introduction of AL into the community. Mothers/ caregivers were said to have used AL in place of the orthodox drugs and herbs reported commonly used prior to study after commencement of AL distribution. The use of CMDs for drug distribution was acceptable to the participants and they were judged to be efficient as they were readily available, distributed correct dose of AL and mobilised the community effectively. AL was perceived to be very effective and no significant adverse event was reported. Major concerns to the sustainability of the program were the negative attitudes of health workers towards discharge of their duties, support to the CMDs and the need to provide CMDs incentives. In addition regular supply of drugs and adequate supervision of CMDs were adviser. In conclusion, our findings showed that the use of AL at home and community level is feasible with adequate training of community medicine distributors and caregivers. Community members perceived AL to be effective thus fostering acceptability. The negative attitudes of the health workers and issue of incentives to CMDs need to be addressed for successful scaling-up of ACT use at community level.

175

IMPLEMENTATION OF ARTEMEMTHER LUMEFANTRINE IN TANZANIA: EARLY LESSONS AND EXPERIENCES

Emmy Metta[1], Seleman Mbuyita[1], Angela Kimweri[1], Elizeus Kahigwa[1], Denise Roth Allen[2], Salim Abdulla[1], S. Patrick Kachur[2]

[1]Ifakara Health Research and Development Centre (IHRDC) Ifakara, Dar es Salaam, United Republic of Tanzania, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States

Tanzania adopted the artemisinin-based combination therapy Artemether - Lumefantrine (ALU) in place of Sulfadoxine Prymethamine in December 2006. We documented the early lessons and experiences at the health facility level 6 months post-implementation of the new policy. Beginning in June 2007, we conducted a rapid assessment at 25 health care facilities (HF) in five districts to document experiences and challenges with implementing the new treatment policy. In-depth interviews with 110 health care workers (HCW), 24 health facility in-charge staff (HFICS), and 12 council health management team (CHMT) members were conducted. Outpatient records were reviewed for prescriptive practices. Inventories and dispensing stations were observed for ALU availability. Exit interviews with 75 clients who had a recent fever episode and focus group discussions with community members were conducted to solicit information on consumer's perceptions and use of the new treatments. Although more than half of HCWs (64/110) had received training on ALU treatment guidelines, interviews with CHMT (12/12) and HFICS (22/24) indicate that many dispensing staff at HFs do not know the correct dosing schedule. One fourth (21/75) of clients who had been prescribed ALU were unable to recite the correct dosage. Directly observed therapy for the first dose of ALU is rarely practiced due to water and utensils shortage. 13/24 HFICS reported experiencing an ALU stock out of at least 1 week in the past 3 months. One third (41/110) of HCWs reported ALU refusal by clients. Inconsistent messages to clients regarding ALU dosage were observed within and between HFs. ALU health education materials were inconsistently distributed among the HFs and communities visited. Community knowledge of ALU dosing and perceptions of ALU effectiveness were mixed, with some worrying that ALU is an experimental drug about which little is known. There is some degree of success with ACT implementation in Tanzania. However, for the effectiveness of ALU to be realized at the community level, several corrective measures to enhance client uptake and adherence should be taken. These include re-training dispensing staff regarding ALU dosing schedules, ensuring a regular supply of ALU to health facilities, and increasing community awareness about ALU and the importance of correct dosing and adherence.

176

CHEMOTHERAPEUTIC EVALUATION OF WHOLE CELL MALARIA VACCINE CANDIDATES

Wilbur K. Milhous[1], Lucia Gerena[2], Mike O'Neil[2], Adriana Ahumada[3], Stephen Hoffman[3]

[1]University of South Florida, Tampa, FL, United States, [2]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [3]Sanaria Inc., Rockville, MD, United States

Sanaria, Inc. and the PATH Malaria Vaccine Initiative have established a one-of-a-kind clinical manufacturing facility to allow scientists to safely rear live, aseptically produced mosquitoes, feed them blood containing the malaria parasite, irradiate the insects to weaken the parasites, and then harvest the parasites from the mosquito salivary glands. The goal is to use parasite clones such as NF54 and 7G8 as the main component of a "whole-parasite" vaccine. In support of quality assurance, the Walter Reed Army Institute of Research has performed culture and susceptibility testing of blood stages of these parasites validating the treatment of choice and phenotypic response to drug therapies to include artesunate, atovaquone/ proguanil (Malarone), chloroquine, cycloguanil, pyrimethamine, mefloquine, proguanil, tafenoquine, primaquine, quinine and doxycycline. Samples from the Master Cell Bank and Working Cell Bank are routinely tested along using control parasite clones W2, D6, TM90C2B and TM91C235. NF54 is a highly drug susceptible parasite with a "wild type" DHFR amino acid sequence similar to D6. Both are markedly susceptible to pyrimethamine and cycloguanil. This is in contrast to the highly resistant (triple-W2 and quad-C2B, 7G8 and TM91 DHFR mutants) which are 160 to 2000 fold more resistant to pyrimethamine. 7G8 is also highly resistant to cycloguanil and chloroquine, but susceptible to mefloquine. Both NF54 and 7G8 are highly susceptible to atovaquone component of Malarone. These finding validate treatment of choice for patients who may develop blood stage parasites derived from NF54 for 7G8 sporozoite inoculua.

177

EFFECTIVENESS OF ORAL QUININE AND ARTEMETHER-LUMEFANTRINE IN THE TREATMENT OF UNCOMPLICATED FALCIPARUM MALARIA IN UGANDAN CHILDREN: A RANDOMIZED TRIAL

Jane W. Achan[1], Daniel Kyabayinze[2], Yeka Adoke[3], Moses Kamya[1], Fred W. Mangeni[1], Umberto D'Alessandro[4], James Tibenderana[2], Ambrose O. Talisuna[3]

[1]Makerere University, Kampals, Uganda, [2]Malaria Consortium, Kampals, Uganda, [3]Ugnada Malaria Surveillance Project, Kampala, Uganda, [4]Institute Of Tropical Medicine, Antwerp, Belgium

Quinine monotherapy is highly efficacious in controlled clinical trials. However, it has several disadvantages such as poor tolerability and a prolonged treatment course. Whether the high efficacy observed under rigorous conditions of clinical trials will be maintained in real life situations is not clear. An open label randomized effectiveness study was conducted at Mulago Hospital in Uganda to compare the parasitological and clinical cure rates and adherence to oral quinine versus artemether-lumefantrine in children aged 6-59 months with uncomplicated malaria. 91 participants were randomized to artemether-lumefantrine administered twice daily for 3 days according to the WHO weight-specific Coartem® blister packs. 86 participants were randomized to oral quinine (10 mg/kg of body weight), administered thrice-daily for 7-days. The first doses of the drugs were administered under the supervision of the study nurse providing at the same time the remaining doses to be taken unsupervised at home. Treatment outcomes were assessed using standardized WHO 28-day clinical and parasitological classifications. 155 (87.6%) participants completed the study and 22 (12.4%) participants withdrew prematurely. Adherence to study drugs was assessed at un-announced home visits on day 7 and reported as the proportion of pills taken divided by the total number of pills prescribed. Mean adherence to artemether-lumefantrine was 95.9 % (SD=13.3) compared to 84.9 % (SD= 20.5) quinine (p-value <0.001). 68 patients (86 %) in the artemether -lumefantrine group had adequate clinical and parasitological responses compared to 50 patients (66 %) in the quinine group (p value =0.003). 18 patients (24%) in the quinine group had late clinical failure compared to 2 patients (2.5%) in the artemether-lumefantrine group (p-value <0.001). 4 patients (4.7%) had late parasitological failure in the quinine group compared to none in the artemether-lumefantrine group (p value = 0.054). There was no difference in the occurrence of severe adverse events in the two groups (overall prevalence 1.7%). Many countries in Eastern and Central Africa that have adopted ACTs as first line regimens have also selected quinine as the second line. However, these findings question the justification for such a decision. The role of oral quinine as second line treatment for uncomplicated malaria needs to be urgently reviewed because another ACT is likely to be a better alternative.

178

AREA UNDER THE EFFICACY CURVE ANALYSIS OF INTRAVENOUS ARTESUNATE IN TREATMENT OF SEVERE MALARIA

Louis Macareo[1], Qigui Li[1], Bryan Smith[2], Mark Polhemus[3], Scott Miller[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [3]United States Army Medical Research Unit - Kenya, Kisumu, Kenya

Most deaths from severe malaria falciparum occur within the first day of presentation, most likely due to multi-organ failure from high parasite burden. Intravenous artesunate has demonstrated superiority over intravenous quinine in reducing this mortality. However, no mortality benefit was noted in the first 24 hours and ideal dosing has not yet been established. Traditional evaluations of parasite clearance time and parasite reduction at 24 or more hours reveal little difference between regimens with such a rapidly acting drug. Data derived from frequent early sampling of 100 subjects enrolled in an ongoing intravenous artesunate dose ranging study will be used to construct an Area Under the Efficacy Curve model to more accurately compare the relative speed of parasite density reduction between regimens. The core study is being conducted in Thailand and Kenya and compares four dosing groups of intravenous artesunate, 1.2mg/kg, 2.4mg/kg and 4.8mg/kg daily for 3 days and 2.4mg initially and again at 12 hours, then daily for 2 more days. Results will be presented at the meeting. The dosing regimen producing the most rapid reductions in parasite density may provide a mechanistic explanation for differences in mortality between dosing regimens and suggest the most clinically efficacious dosing regimen.

179

POPULATION PHARMACOKINETICS OF CHLORPROGUANIL-DAPSONE-ARTESUNATE AND THEIR MAJOR METABOLITES IN CHILDREN AND ADULTS DURING TREATMENT OF ACUTE UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

Ann K. Miller[1], Mita Thapar[2], Siobhan Hayes[2], Ammar Qureshi[3], Cletus Ugwuegbulam[3], Li-Ean Goh[3], Allan Pamba[3]

[1]GlaxoSmithKline Pharmaceuticals, King of Prussia, PA, United States, [2]ICON Development Solutions, Marlow, Buckinghamshire, United Kingdom, [3]GlaxoSmithKline Pharmaceuticals, Greenford, Middlesex, United Kingdom

There are limited pharmacokinetic (PK) data available for chlorproguanil (CPG)-dapsone (DDS)-artesunate (ART) in patients with malaria, particularly for ART due to its rapid metabolism. As part of a phase III trial comparing the safety and efficacy of the combination of CPG-DDS-ART (CDA) and artemether-lumifantrine for treatment of acute uncomplicated *Plasmodium falciparum* malaria, the population PK of CDA are being characterized in approximately 900 children from six African countries. Children (aged ≥ 1 to 14 years old) were randomized to CDA received 2/2.5/4 mg/kg once daily for 3 days. Blood samples (3-5 per child) were collected to evaluate the population PK of CPG, DDS and ART and their major active metabolites chlorcycloguanil (CCG), and dihydroartemisinin (DHA). The plasma concentrations (Cp) of the three drugs and their metabolites were determined by LC-MS/MS. These data were combined with Cp from 53 healthy volunteers (HV) and 115 adult patients from previous studies. Population PK models are being developed from these data and will be presented. These models will be used to examine the effect of covariates (e.g. age, weight, gender, patients vs. HV, G6PD genotype/phenotype, concomitant medications, and selected lab values) on the population PK parameters. The correlation between the individual drug exposures and efficacy (i.e. parasitological cure at Day 28) and safety parameters (e.g. haematological adverse events) will be explored.

180

FOUR COMMUNITY-BASED NEGLECTED INFECTIOUS DISEASE COLLABORATIVE DRUG DISCOVERY CASE STUDIES

Barry Bunin, Moses Hohman, Sylvia Ernst, Kellan Gregory

Collaborative Drug Discovery, Burlingame, CA, United States

Case studies from scientists working in secure collaborative groups to rapidly develop drug candidates for commercial and humanitarian markets will be presented. In the first case study, the discovery of alternatives to Verapamil, a known chemosensitizer to overcome both tumor and malaria resistance, will be presented using novel collaborative drug discovery technologies. A chemosensitizers addressing chloroquine resistance was identified combining results from the University of Cape Town (South Africa) with structurally related compounds from the University of California at San Francisco (USA) and similar FDA/Orphan (courtesy Dr. Lipinski) approved drug compounds. The new collaborative technology allows researchers to build up networks of technical experts around therapeutic or target areas thus facilitating discovery of new drug candidates. Other case studies include: a Malaria Computational and Experimental around large set of historical small molecule animal SAR data

case study (UNC, St. Jude), a Malaria UGI-4CC Open Collaboration case study (Drexel-Indiana-UCSF), a Tuberculosis Public Private Partnership case study (TAACF, Lilly, Cornell), and a GPCR gene-family wide Ki community Database (PDSP, UNC). The community-based platform is currently being used securely share sensitive SAR data to help develop new treatments for neglected infectious diseases such as malaria, Chagas Disease, and African Sleeping Sickness.

181

THAI TRADITIONAL MEDICINE PLANT, *TINOSPORA CRISPA*, AFFECTS THE *PLASMODIUM* INFECTIVITY *IN VIVO*

Thanaporn Rungruang[1], Thidarut Boonmars[2]

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

Malaria is one of the most infectious diseases that leads to death and illness of the world population annually. Nowadays, malaria prevalence is likely to continue increasing since the disease is resistant to the available and safe antimalarial drugs. Thus, the discovery of effective novel drugs is very much concerned. Within a circumstance of growing of malaria resistance, the development and promotion of traditional medicines, which widely believe among the people especially the villagers and local people to be safe and also effective, is the alternative choice to malaria treatment. Thailand is one of the tropical countries which people suffer from malaria, besides, having various kinds of plants which found to have the properties to be phytomedicines. Furthermore, little knowledge has been reported. Hence, investigating the possible Thai traditional plant is interested. In our studies, we examined the effect of ethanol extract of *Tinospora crispa*, the Thai traditional plant, to the malaria infectivity *in vivo*. Mice were divided into control and experimental groups. All mice were inoculated with *Plasmodium yoelii* before treatment of varying doses of *T. crispa* Parasitemia was checked by Giemsa staining daily. Comparing to the control that was given ethanol only, the higher doses of crude extract of *T. crispa* we administrated, the lower parasite infection in the mice occurred consequently the mice lived longer. In the conclusion, *T. crispa* showed antimalarial effect *in vivo* in dose dependent manner.

182

PHARMACOKINETIC EVALUATION OF ARTESUNATE FOLLOWING SINGLE INTRAVENOUS INJECTION OF 0.5, 1, 2, 4, AND 8 MG/KG IN NORMAL, HEALTHY VOLUNTEERS

Qigui Li[1], Louis R. Cantilena[2], Kevin J. Leary[2], George A. Saviolakis[2], R. Scott Miller[1], Victor Melendez[1], Peter J. Weina[1]

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

Safety, tolerability, and pharmacokinetics (PK) of artesunate (AS) were evaluated after single doses of 0.5, 1, 2, 4, and 8 mg/kg over a 2 minute intravenous infusion in 40 healthy volunteers. Plasma samples for measurement of AS and dihydroartemisinin (DHA, active metabolite of AS) were analyzed by LC-MS/MS using validated analytical procedures. Results revealed a dose-dependent increase in mild adverse events; specifically, altered taste and decreasing reticulocyte counts. This study agrees with published literature showing that AS is rapidly converted to DHA in healthy subjects. The DHA could be detected in all volunteers' plasma for up to 6 hours after the initial injection, while the parent drug (AS) was undetectable at time-points as early as 1-2 hr post infusion. Both drugs were rapidly cleared with an overall mean elimination half-live ranging from 0.12-0.24 hr for AS and 1.15-2.37 hrs for DHA. Concentration versus time data confirmed the pharmacokinetic analyses of both model-dependent and model-independent PK analyses for AS and DHA. Although the plasma concentration of DHA was higher than that of AS ($AUC_{DHA/AS}$ ratio = 1.12-1.87), the peak concentration of AS was higher than that of DHA ($C_{max\ DHA/AS}$ ratio = 2.8-4.51). The AUC and C_{max} of both AS and DHA were increased proportionally (r^2 = 0.969-0.997) with ascending doses of AS. DHA is slightly more potent to AS *in vitro*, but it is also the only artemisinin derivative that has broader activity against all stages of blood merozoites. Therefore, the effectiveness of AS has been attributed due not only to the high initial C_{max} of parent drug, AS, but also to its rapid hydrolysis to DHA. The study provides the first in human data of an US GMP formulation of the candidate antimalarial.

183

COMPARTMENTAL MODEL-DEPENDENT ANALYSIS OF PHARMACOKINETIC DATA OF ARTESUNATE FOLLOWING SHORT-TERM INFUSION IN HEALTH VOLUNTEERS

Qigui Li, Peter J. Weina

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

Compartmental analysis (CA) and non-compartmental analysis (NCA) lead to almost identical estimates of key parameters commonly used for pharmacokinetic (PK) evaluation on many clinical trials. In our phase 1 study with intravenous artesunate (AS), both CA and NCA were performed following a single and short-term infusion (2 minutes). Based on a two-compartment with IV-infusion model (WinNonlin model 10), the C_{max} ranged from 19420 to 83340 ng/ml and AUC of 1595-6994 ng·h/ml were estimated. However, NCA evaluation (WinNonlin model 202, IV-infusion) resulted in a 38% to 75% decrease in values for these calculations for these parameters; the C_{max} of 4827-21098 ng/ml and AUC of 969-4331 ng·h/ml. In the NCA method, the trapezoidal method was used to calculate the AUC and the first experimental value (at 7 min) was set to estimate the C_{max}. For that reason, the model 202 did not estimate a real C_{max} at 2 minutes (the end time of the infusion), and it did not as accurately estimate the AUC from 0 to 7 minutes due to the difference in the C_{max}. Use of the CA model 10 seems to be a more suitable system to estimate the true C_{max} at 2 minutes and the precise AUC after short-term infusions as in our study. Although both of these approaches for determining the PK data are acceptable to the FDA with many drugs in normal cases, the down values (38-75%) of AUC and C_{max} found in present report with NCA model may be misleading in the evaluation of AS, and may explain some of the very high variability seen in the literature from clinical trials. Therefore, we believe that the compartmental (model-dependent) PK analyses should be considered in the clinical trial evaluation of very short acting drugs (such as with AS) following short-term infusion.

184

EFFECTS OF *PLASMODIUM BERGHEI* AND *P. YOELII* SPOROZOITE NUMBER ON *IN VIVO* INFECTION RATES AND TIME TO PATENCY IN C57BL/6 MICE

Jing Zhang, Lisa H. Xie, Qiang Zeng, Erin Peacock, Qigui Li, Michael T. O'Neil

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

Mouse-malaria liver stage models are widely used for initial *in vivo* drug and vaccine efficacy studies. A well-validated model requires 100% infection in untreated control animals with a consistent onset to patency. *Plasmodium species*, sporozoite number, and mouse strain greatly influence infection rate and onset of patency. Our laboratory evaluated the effect of *P. berghei* (ANKA) and *P. yoelii* (17NXL) sporozoite number on infection rates and time to patency in C57BL/6 mice. Intravenous (IV) injection of 10,000 *P. berghei* or *P. yoelii* sporozoites produced blood-stage infections in 57/57 and 52/52 C57BL/6 mice, respectively. The time to patency was 4.33 (± 0.58) days for *P.berghei* infections and 8.33 (± 0.65) days for *P. yoelii* infections. Additional *P. berghei* studies showed that IV injection of 4,000 sporozoites produced 100% infection in C57BL/6 mice (20/20) with no significant delay to patency (5 days). The minimum number of *P. berghei* and *P. yoelii* sporozoites required to initiate an infection in 100% of C57BL/6 mice was 150 and 100, respectively. The lower sporozoite inoculums resulted in delays to patency in both *P. berghei* (day 7) and *P. yoelii* (day 10) infections. Based on our results, *P. berghei* and *P. yoelii* provide similar infection rates at comparable sporozoite

numbers. *P. berghei* sporozoite-induced infections always became patent days earlier than *P. yoelii* at the same sporozoite inoculum.

185

COMPARISON OF HPLC WITH ELECTROCHEMICAL DETECTION AND LC-MS/MS FOR THE SEPARATION AND VALIDATION OF ARTESUNATE AND DIHYDROARTEMISININ IN ANIMAL AND HUMAN PLASMA

Yuanchao C. Gu, Qigui Li, Victor Meledez, Peter J. Weina

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

High-performance liquid chromatography with reductive electrochemical detection (HPLC-ECD) method has been used for assaying artemisinins in Walter Reed Army Institute of Research since 1985. Although the methods have been remarkably improved, tandem mass spectrometry (LC-MS/MS) systems with significant advantages have gradually replaced HPLC-ECD to analyze artesunate (AS) and dihydroartemisinin (DHA) in plasma. In the present study, the two methods were evaluated for linearity, quantitation limits, selectivity, precision, and accuracy. New HPLC-ECD systems consisted of Agilent HPLC and ESA electrochemical detector, which uses porous graphite electrodes. This type of probe can be used to measure reductive agents for extended periods before any electrode maintenance is required. To validate the two approaches, a steady-state pharmacokinetic parameters and tolerant studies of AS dissolved in 0.3M PBS were evaluated in 12 male beagle dogs following a daily intravenous administrations of the drug at 20 mg/kg for 3 consecutive days. Correlation plots of plasma concentration yielded r^2 values of 0.932-0.976, indicating a high degree of linear correlation between HPLC-ECD and LC-MS/MS determinations for both AS and DHA. The new HPLC-ECD performed well in terms of various validation parameters, and showed a good agreement with the LC-MS/MS when used to quantify drug levels in animal and human plasma. However, the major benefit of LC-MS/MS was that it required only one-tenth the plasma volume needed by HPLC-ECD assay. The data obtained will help with the design and implementation of future PK studies.

186

TISSUE DISTRIBUTION, METABOLIC PROFILES AND PROTEIN BINDING OF [^{14}C] DIHYDROARTEMISININ FOLLOWING SINGLE INTRAVENOUS ADMINISTRATION IN RATS

Lisa H. Xie, Qigui Li, Jing Zhang, Peter J. Weina

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

This study reports the tissue distribution, pharmacokinetics, mass balance, and elimination of [^{14}C] dihydroartemisinin (DHA) following single intravenous administration in rats. Protein binding was performed with rat and human plasma. Plasma, urine, and feces samples were collected up to 192 hr and the drug concentrations were non-classically obtained from measurements of total radioactivity to determine the contribution by the parent and metabolites to the total dose of drug injected. Metabolism of DHA was observed with high excretion via bile (mostly in a conjugated form) and approximately 89-95% dose of all conjugations were accounted for in blood, urine and feces. However, the majority of elimination of [^{14}C] DHA was through urinary excretion (52% dose). The mean terminal half-lives of plasma and blood radioactivity (75.57-122.13 h) were significantly prolonged compared with that of unchanged DHA (1.03 h). We hypothesize this was due to a possible drug re-absorption in the intestines and enterohepatic circulation as suggested by multiple peak concentrations in blood, plasma and tissues. The long lasting metabolites of DHA (> 192 hr) may be also related to enterohepatic circulation. Drug was widely distributed after 1 hr. After 24 hrs, the radioactivity rapidly declined in all tissues except spleen until 96 hrs. Only 0.81% of the total radioactivity was detected in brain tissue. DHA revealed a high binding capacity with both rat and human plasma proteins (76-82%). The concentration of total radioactivity in the plasma fraction was less than a half of that in blood total. Higher binding to RBCs and a longer half-life of DHA may relate to its powerful antimalarial activity.

187

ANTIPLASMODIAL BENZOPHENONES FROM *ENDODESMIA CALOPHYLLOIDES* (GUTTIFERAE)

Erasmienne T. Ngouamegne[1], René S. FONGANG[1], Silvere Ngouela[1], Fabrice F. Boyom[1], Michel Rohmer[2], Etienne Tsamo[1], Jiri Gut[3], Philip J. Rosenthal[3]

[1]University of Yaounde [1], Yaounde, Cameroon, [2]Université Louis Pasteur, Strasbourg, France, [3]University of California, San Francisco (UCSF), San Francisco, CA, United States

New effective antimalarial drugs are urgently needed. To achieve the goal of discovering such agents, every resource must be investigated, including plant-derived metabolites. The present work describes the isolation, structural elucidation and antiplasmodial activity of benzophenones from *Endodesmia calophylloides* stem bark. Chromatographic separation using organic solvents afforded six benzophenones of the guttiferone family. The structures of these compounds were elucidated by means of spectroscopic methods (UV, IR, MS, ^{1}H and ^{13}C NMR, 1D and 2D). The structures of known compounds were further confirmed by comparison of their spectroscopic data with those from the literature. The antiplasmodial activity was evaluated *in vitro* against the W2 strain of *Plasmodium falciparum.* All the benzophenones showed significant potencies with IC_{50} values ranging from 1.5 to 10 µM. They exerted no toxicity against erythrocytes at antiplasmodial concentrations. The results achieved highlight benzophenones as a potential source of antimalarial leads compounds.

188

CLINICAL SAFETY AND DRUG INTERACTIONS WITH 8-AMINOQUINOLINES

Hla Y. Myint[1], Colin Ohrt[1], Aruna Sampath[2], Larry Walker[2], Nick White[3], Alan Magill[1]

[1]Walter Reed Army Institute Research, Silver Spring, MD, United States, [2]University Of Mississippi, University, MS, United States, [3]Mahidol Oxford Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Primaquine, pamaquine and tafenoquine are 8-aminoquinoline antimalarials with clinical data available. They are all active against *Plasmodium vivax* hypnozoites and stage 5 *P. falciparum* gametocytes making them highly desirable for malaria prevention, control and elimination. A major downside with this class of drugs is that patients with glucose 6 phosphate dehydrogenase (G6PD) deficiency develop hemolytic anemia. The lack of predictive preclinical models has impaired us from selecting a member of this class that does not have this risk. An advisory meeting with 61 individuals from 13 institutions was held in Bethesda, Maryland on January 15-16, 2008. The outcome of this meeting has lead to a non-hemolytic 8-aminoquinoline consortium evaluating *in vitro* and *in vivo* models for their predictive ability for G6PD deficiency. To support this effort, all of the premedline literature and unpublished data is being reviewed. An extensive literature on the safety of pamaquine and primaquine in humans has been identified. In the era before primaquine, a number of deaths were reported with pamaquine. Pamaquine needed to be coadministered with quinine or quinacrine to achieve any antihypnozoite efficacy. Doses much above that causing hemolytic anemia were needed to achieve efficacy. More than 100 aminoquinolines were studies in humans before primaquine as downselected in the 1950s. No deaths have been reported with primaquine despite extensive use without screening for 50 years. It has a much improved therapeutic index in comparison with pamaquine. In addition, clear-cut evidence of the potentiation of primaquine with quinine and with chloroquine was found in human challenge studies in the 1950's. In conclusion, a throughout understanding of the clinical interaction and safety data of

existing 8-aminoquinolines may help us identify a new non-hemolytic 8-aminoquinoline.

189

EVIDENCE OF HEMOLYTIC ANEMIA IN TAFENOQUINE AND PRIMAQUINE ANIMAL STUDIES

Yarrow Rothstein[1], James Myer[2], Geoffrey Dow[1], Peggy Wasson[1], Larry Walker[1], Alan Magill[1], Colin K. Ohrt[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]GlaxoSmithKline, Research Triangle Park, NC, United States

Tafenoquine (TQ) and primaquine (PQ) are both antimalarial drugs in the 8-aminoquinoline class. TQ is still under development, while PQ has been approved since the 1950s. To date, preclinical models predictive of risk in G6PD-deficiency have not been identified. Preclinical toxicology studies were examined to determine if the hemolytic pattern in normal animals may predict risk of hemolysis in G6PD-deficient humans. Eleven animal studies: three PQ studies (one each of dog, monkey, and rat) and eight TQ studies (four dogs, two monkeys, and two rats), were examined to study different clinical indicators of hemolytic anemia. Data from the following nine hemolytic and biochemistry parameters were included: erythrocyte count, hemoglobin, hematocrit, haptoglobin, Heinz Bodies, nucleated red blood cells, reticulocytes, lactate dehydrogenase (LDH), and methemoglobin. Data were entered into Microsoft Excel 2003 and graphed to visualize trends. The studies ranged from 28 days to one year in length. Day 14 was the primary time point of interest. In the examined studies, the following decreasing dose-response trends were observed: erythrocytes decreased in six out of ten studies. The following increasing trends were observed: haptoglobin was found to increase in all six studies; Heinz bodies increased in four out of five studies; methemglobin increased in ten out of eleven studies; nucleated red blood cells increased in three out of five studies; reticulocytes increased in seven out of nine studies. The following varied trends were observed: hematocrit and hemoglobin decreased in five out of eleven studies, with one study exhibiting increases in both parameters; LDH had increased in two out of five studies, but was decreased in one study. Analysis of additional studies including those in normal human volunteers is underway. Normal animal models appear to show several tends consistent with hemolytic anemia. Multiple factors appear to be affecting some of these biomarkers. After our analysis is complete, we hope to be able to prospectively validate the parameters that appear to be most promising.

190

A NOVEL CHEMOTYPE WITH POTENT ACTIVITY AGAINST *PLASMODIUM FALCIPARUM* AND *P. BERGHEI*

Sameer Urgaonkar[1], Ralph Mazitschek[1], Cassandra Celatka[2], Joseph Cortese[3], Hanlan Liu[2], Mandy Cromwell[2], **Robert H. Barker**[2], Miryam Garcia Rosa[4], Adelfa Serrano[4], Dyann F. Wirth[5]

[1]Broad Institute of Harvard and MIT, Cambridge, MA, United States, [2]Genzyme Corporation, Waltham, MA, United States, [3]Broad Institute of Harvard and MIT, Waltham, MA, United States, [4]University of Puerto Rico-School of Medicine, San Juan, PR, United States, [5]Harvard School of Public Health, Boston, MA, United States

The compound (Genz-644442) described here was initially identified as "hit" from an ongoing high throughput screening campaign being done by the Broad Institute and Genzyme Corporation in collaboration with Malaria Medicines Ventures. Genz-644442 has IC_{50}'s of 435, 520 and 796 nM against *Plasmodium falciparum* strains 3D7, HB3 and Dd2 respectively. *In vitro* absorption, distribution, metabolism, and excretion (ADME) studies demonstrated high metabolic stability in human and rat hepatocytes, lack of human CYP inhibition, moderate lipophilicity with a Log D of 3.0, good solubility and permeability characteristics. The compound is well-tolerated in mice when dosed over 4 days at doses up to 50 mg/kg/day. Pharmacokinetic (PK) studies in mice showed that Genz-644442 had a terminal $T_{1/2}$ of 7.45 hr and plasma levels of 30x the IC_{90} when dosed intraperitoneally at 50 mg/kg. When dosed at 50 mg/kg/day for 4 days in mice infected with *P. berghei*, no parasites were detected on Day 4 post infection; however, parasites recrudesced by Day 9. Preliminary patch-clamp experiments showed that hERG channel inhibition is >25 μM. A novel chemical methodology was developed to synthesize analogs, since published methods were not amenable to analog library synthesis. Approximately 35 analogs have been synthesized. Based on the gained SAR data, we have continuously optimized the series to develop compounds with IC_{50}'s of approximately 10 nM.

191

MOLECULAR SURVEILLANCE OF DRUG-RESISTANCE ASSOCIATED MUTATIONS OF *PLASMODIUM FALCIPARUM* IN TWO DISTINCT GEOGRAPHICAL AREAS OF NIGERIA USING REAL TIME PCR TECHNIQUES

Daniel O. Ojurongbe[1], Segun I. Oyedeji[2], Wellington A. Oyibo[3], Taiwo A. Ojurongbe[4], Adetayo F. Fagbenro-Beyioku[3], Peter G. Kremsner[5], Juergen J. Kun[5]

[1]Ladoke Akintola University of Technology, Osogbo, Osun State, Nigeria, [2]Bells University, Sango Ota, Nigeria, [3]University of Lagos, Lagos, Nigeria, [4]Justus Liebig University, Giessen, Germany, [5]University of Tuebingen, Tuebingen, Germany

Malaria continues to be one of the most important infectious diseases in the world, affecting mainly the tropics and subtropics and drug resistance against *Plasmodium falciparum* has been recognized as the crucial obstacle to curbing mortality and suffering from malaria. We therefore determined the baseline distribution of Pfcrt, Pfmdr1 genes mutation associated with resistance to chloroquine and Dhfr genes association with resistance to sulfadoxine-pyrimethamine in *P. falciparum* isolates collected from two geographically distinct areas of Nigeria using novel real time PCR techniques. The combine prevalence of Pfcrt T76 mutation in the two sites was 93.3% with 86% from Osogbo compared to 93% from Lafia. The difference was not statistically significant (P=0.4453). Sequencing analysis of the Pfcrt K76T haplotype (amino acids 72_76) revealed CVIET as the only resistance haplotype present in the two areas. The frequency of the Pfmdr1 gene was higher in Lafia (39%) compared to Osogbo (26%) and the combine prevalence from the two sites was 45.5% but the difference was not statistically significant (P=0.6604). The prevalence of the Dhfr triple mutant alleles was high in the two locations. The Osogbo *vs* Lafia prevalence for Dhfr mutations was 84% *vs* 91%, 88% *vs* 87% and 96% *vs* 96% for I51, R59 and N108 respectively. None of the samples from the two locations had the T108 mutation. The combine prevalence of Pfcrt and Pfmdr1 in Osogbo and Lafia was 44.2% with a risk ratio of 0.4164 while the combine prevalence of Pfcrt, Pfmdr1 and Dhfr was 40.4% with risk ratio of 1.081. These results strongly suggest the widespread distribution of CQ and pyrimethamine resistance without any marked distinction between the two locations. In addition, the result confirms the usefulness of RT-PCR in the detection of *P. falciparum* alleles associated with drug resistance. Fret hybridization probe method successfully detected Pfcrt *and* Pfmdr1 *alleles* while LNA dual labeled probe assay successfully detected the triple Dhfr mutations. The assays were sensitive and specific with low per test cost, fast, easily automated and well-suited for large scale epidemiological studies.

192

ADAPTATION OF A MULTIDRUG RESISTANT C2A CLONE OF *PLASMODIUM FALCIPARUM* FROM THAILAND TO *AOTUS LEMURINUS LEMURINUS* MONKEYS AND ITS PRELIMINARY *IN VIVO* ANTIMALARIAL DRUG SENSITIVITY-RESISTANCE PROFILE

Nicanor III Obaldia[1], Wilbur K. Milhous[2], Dennis E. Kyle[2]

[1]Tropical Medicine Research/Gorgas Memorial Institute, Panama, Panama, [2]Department of Global Health, College of Public Health, University of South Florida, Tampa, FL, United States

Multidrug resistant (MDR) *Plasmodium falciparum* parasites are increasing at an alarming rate in terms of both prevalence and severity. These strains have been detected along the Thai-Myanmar and Thai-Cambodian borders and its drug resistance profile have being studied in humans *in vivo* and *in vitro*, as reported previously. With the emergence of multidrug-resistant falciparum malaria in different parts of the world, new drugs and drugs in combination are urgently needed. The importance of a reliable animal model for *in vivo* testing of new antimalarial compounds or combinations thereof against these MDR strains could not then be overemphasize. The human *P. falciparum*/Aotus monkey model is an excellent pre-clinical model that have been used extensively for testing the efficacy of new antimalarial compounds since the first demonstration in 1966 by Martin Young, working at Gorgas Memorial Laboratory in Panama, that *Aotus trivirgatus* could be infected with human plasmodia. Herein, we described the adaptation of a C2A clone of *P. falciparum* from Thailand to *Aotus l. lemurinus* monkeys and present preliminary *in vivo* data on its drug sensitivity-resistance profile against artemisinin derivatives (artelinic and artesunic acid), mefloquine, quinine, an experimental DHFR inhibitor and atovaquone/proguanil (malarone). This model will allow testing the efficacy of new therapies against MDR parasites.

193

THE EFFICACY OF MALARTIN/SULPHADOXINE-PYRIMETHAMINE IN THE TREATMENT OF UNCOMPLICATED FALCIPARUM MALARIA IN A RURAL SETTING OF THE MOUNT CAMEROON REGION

Helen K. Kimbi[1], Theresa Nkuo-Akenji[1], Mesame Ntoko[1], Nelson N. Ntonifor[1], Emmaculate Lum[1], Bernice F. Muh[1], John Egbe[2]

[1]University of Buea, Buea, Cameroon, [2]Christian Medical Foundation, Dibanda, Buea, Cameroon

Artemisinin derivatives are now the most potent and rapidly acting antimalarial drugs to which the malaria parasite has not yet developed resistance. The aim of this study was to assess the *in vivo* efficacy and tolerability of a combination of Malartin (an artesunate) and sulphadoxine-Pyrimethamine (Fansidar) in the treatment of uncomplicated falciparum malaria in a rural setting of the Mount Cameroon Region between December 2006 and August 2007. A total of 197 subjects (≥ 1 year of age) were recruited into the study, after meeting the inclusion criteria for the study. They were then given the appropriate doses of the drugs for 3 days and follow-up was done on days 3, 7 and 14. Only 174 of the subjects were successfully followed-up (174 males and 100 females). A combination of Malartin and Fansidar was found to be effective in clearing parasitaemia, fever and improving on the anaemia status of the patients. Clinically, the overall success rate (ACPR) was 92.5% (161/174), and therapeutic failures (ETF, LCF and LPF) were experienced in 07.5% (13/174) of the subjects. Parasite density generally decreased during the follow-up period in the different age groups and sexes, but the difference was not significant (except on day 0 for the age groups and day 7 for the sexes). The prevalence of anaemia (PCV <31%) was 23.0% at enrolment and decreased to 10.0% on day 14, and the difference was significant ($p<0.05$) on all days of follow-up, except on day 7. The drug combination was well tolerated and did not give rise to any serious side effects. The most frequent side effects were fatigue, fever and dizziness. Most of the side effects were self-limiting as most of them disappeared by day 14.

194

SIMULTANEOUS IDENTIFICATION OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) IN *PLASMODIUM FALCIPARUM DHFR, PFDHPS, PFCRT* AND *PFMDR1* GENES USING A MULTIPLEXED FLEXMAP™ MICROSPHERE-BASED MINISEQUENCING ASSAY

Carola J. Salas, David J. Bacon

U.S. Naval Medical Research Center Detachment, Lima, Peru

There is an ever increasing need for development of new technologies that allow rapid, cost-effective, high-throughput detection of polymorphisms associated with drug resistant in *Plasmodium falciparum* Here we present our progress in the development of a mini-sequencing procedure using FlexMap™ technology to facilitate the simultaneous identification of known SNPs associated with *in vivo/in vitro P. falciparum* antimalarial drug resistance. Using *P. falciparum* genomic DNA, we prepared five standard PCR reactions to amplify *Pfdhfr, Pfdhps*, *Pfcrt and Pfmdr1* genes. SNP specific primers containing a 5' capture sequence were designed so that the terminal dNTP of the primer anneals directly over the SNP. Two primers were designed for each SNP; one primer terminates with the wild type and one terminates with the mutant nucleotide. Fluorescently labeled allele-specific extension products (ASEP) were generated using the PCR amplified gene, SNP primers and a standard PCR reaction mixture containing biotin-labeled dCTPs and unlabeled dATPs, dGTPs and dTTPs. Following the extension step, the tagged ASEPs were captured using fluorescent microsphere containing DNA complimentary to the tags. After incubation with streptavidin-R-phycoerythrin the reactions were analyzed using the Bio-Rad Bio-Plex™ system, a multiplexed microsphere-based suspension array platform capable of analyzing and reporting up to 100 different reactions in a single reaction vessel. We successfully prepared four different ASEP reactions as well a as well a single 5X multiplex reaction that can accurately identify a total of 45 microspheres corresponding to 45 SNPs of interest. This technology provides a new platform for high-throughput nucleic acid detection, it is robust and qualitative and due to the cost savings associated with this procedure, it could replace traditional DNA sequencing as the gold standard for molecular markers determination in *P. falciparum*.

195

THERAPEUTIC EFFICACY OF ARTEMETHER/LUMEFANTRINE AND AMODIAQUINE/ARTESUNATE FOR THE TREATMENT OF UNCOMPLICATED FALCIPARUM MALARIA IN CHILDREN AGED 6-59 MONTHS AT MKUZI AND UJIJI SENTINEL SITES IN TANZANIA

Samwel H. Sembuche[1], Johannes B. Kataraihya[2], Ezekiel K. Malecela[1], Juma A. Akida[1], Vito Baraka[1], Deo Maiga[1], Filbert Francis[1], Alex Mwita[3], Renata Mandike[3], Deus Ishengoma[1], Watoky M. Nkya[4], Martha M. Lemnge[1]

[1]National Institute for Medical Research, Tanga, United Republic of Tanzania, [2]Bugando Medical Centre and BUCHS, Mwanza, United Republic of Tanzania, [3]Ministry of Health, NMCP, Dar es Salaam, United Republic of Tanzania, [4]Mbeya Consultant Hospital, Mbeya, United Republic of Tanzania

A study to assess the efficacy of artemether/lumefantrine (Coartem®) and amodiaquine plus artesunate (AQ+AS) was conducted in 2007, at Ujiji (Kigoma) and Mkuzi (Muheza) sentinel sites in Tanzania. The main study aim was to obtain data on *Plasmodium falciparum* response to the 2 drugs following change in National Antimalarial Drug Policy. The study involved 28-day follow-up period. Children aged 6-59 months attending Mkuzi and Ujiji Rural Health Centres were screened for malaria parasites and anaemia. Those with *P. falciparum* monoinfection who met other inclusion criteria were randomized to receive either Coartem or AQ+AS. Filter paper

bloodspots were collected for PCR analysis. Coartem® was given to 131 and 102 cases at Mkuzi and Ujiji and AQ/AS to 129 and 103 cases at the 2 sites respectively. Both drugs were given according to weight and under supervision. Significantly higher (p<0.0001) malarial parasite prevalence was found among those screened at Mkuzi (58.1%) compared to Ujiji (26.0%). In the Coartem® arm, adequate clinical and parasitological response (ACPR) by day 28 was 62.4% at Mkuzi and 84.7% at Ujiji. In the AQ+AS arm, ACPR by day 28 was 38.7% and 78.8% at Mkuzi and Ujiji respectively. Total treatment failures with Coartem by day 28 (not PCR corrected) were 37.6% at Mkuzi and 15.3% at Ujiji whilst failures in AQ+AS arm were 61.3% at Mkuzi and 21.2% at Ujiji. In the Coartem arm, ACPR by day 14 was 95.9% at Mkuzi and 97.8% at Ujiji. ACPR in AQ+AS arm by day 14 was 79.5% and 92.3% at Mkuzi and Ujiji respectively. Total treatment failure with Coartem by day 14 was 4.1% at Mkuzi and 2.2% at Ujiji; whilst failure in AQ+AS arm was 20.5% at Mkuzi and 7.4% at Ujiji. Despite similar ACPR with Coartem on day 14 at both sites, significantly higher (p=0.007) ACPR was seen at Ujiji (84.7%) compared to Mkuzi (62.4%). Significant improvement in mean Hb levels was seen on both days 14 and 28 in both treatment arms at both sites; but levels at day 28 were higher than day 14. High failures by day 28 despite excellent response by day 14 might be due to new infections; PCR corrected data will resolve this. Marked Hb recovery at both day 14 and 28 suggests malaria was the major cause of the initial anaemia. Data obtained here will be fed into the National Malaria Control Programme database for future use in reviewing anti-malarial drug policy in Tanzania.

196

PHENOTYPING SENSITIVITY AND RESISTANCE TO CHLOROQUINE IN *PLASMODIUM VIVAX*: STUDIES AT SENTANI, NORTHEASTERN PAPUA, INDONESIA

Puji BS Asih[1], John Leake[2], Yohanna Sorontou[3], Robert Sauerwein[4], Joseph Vinetz[2], Din Syafruddin[1], J. Kevin Baird[5], J. Kevin Baird[6]

[1]Eijkman Institute for Molecular Biology, Jakarta, Indonesia, [2]Department of Internal Medicine, University of California at San Diego, CA, United States, [3]School of Medicine, Cendrawasih University, Papua, Indonesia, [4]Department of Medical Microbiology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands, [5]Nuffield Department of Clinical Medicine, Oxford University, Oxford, United Kingdom, [6]Eijkman-Oxford Clinical Research Unit, Jakarta, Indonesia

Chloroquine remains the first-line therapy for treatment of acute vivax malaria after 60 years of continuous use. Chloroquine-resistant *Plasmodium vivax*, first recorded in 1989, is now known to be a severe problem in much of eastern Indonesia, with more than half of infections exhibiting therapeutic failure. In support of efforts aimed at searching for mutations linked to resistance to chloroquine, we evaluated the therapeutic response to chloroquine in 73 subjects naturally infected by *P. vivax* in northeastern Papua, Indonesia. We phenotyped these infections as susceptible or resistant to chloroquine using a 28-day *in vivo* test format. 18 (25%) subjects had infections presumptively classified as resistant on the basis of persistent or recurrent parasitemia. 3 (17% of resistant infections) subjects had persistent parasitemia at Day 4 or had recurrent parasitemia by Day 7 and were considered early treatment failures. 7 (39%) had recurrent parasitemia by Day 14, and 8 (44%) Day 28. 55 (75%) subjects had no recurrent parasitemia diagnosed microscopically by Day 28 and were presumptively classified as having infections sensitive to chloroquine. The final step in the phenotyping process involves evaluating CQ+DCQ levels on day of recurrent parasitemia, as well as nested PCR on Day 28 samples from infections presumptively classified as sensitive. Those data are in process. Our study affirms prevalent resistance to chloroquine in this region of Indonesia, and we describe a simple, standardized system for phenotyping *P. vivax* infections as a first step in conducting genetic analysis of parasite genotypes linked to therapeutic responsiveness.

197

IDENTIFICATION OF MOLECULAR MARKERS IN *PLASMODIUM FALCIPARUM* ISOLATES ASSOCIATED TO MEFLOQUINE AND ARTESUNATE DRUG RESITANCE IN THE PERUVIAN AMAZON BASIN

Valeria R. Soberon[1], Carola J. Salas[1], Meddly L. Santolalla[1], Andrea M. McCollum[2], Venkatachalam Udhayakumar[2], Carmen M. Lucas[1], David J. Bacon[1]

[1]U.S. Naval Medical Research Center Detachment, Lima, Peru, [2]Centers for Disease Control and Prevention, Division of Parasitic Diseases, Malaria Branch, Atlanta, GA, United States

Plasmodium falciparum malaria was treated in the Peruvian Amazon Basin for over 40 years with Chloroquine (CQ). Increased levels of treatment failure due to CQ resistance in 1997 forced the Peruvian Ministry of Health to change the first line therapy to Sulfadoxine-Pyrimethamine (SP). After only a few years SP resistance had developed. Therefore an Artemisinin Combination Therapy (ACT) based on Artemisin (AS) and Mefloquine (MQ) was implemented in 2003. The *Plasmodium falciparum multidrug resistance gene 1* (*Pfmdr1*) has been a candidate for CQ resistance and other chemically unrelated drugs, MQ and AS included. Recently, the *Plasmodium falciparum ATPase 6* gene (*Pfatp6*) has been proposed as the molecular target for Artemisinin based compounds. This study attempts to identify potential molecular markers within these genes associated with resistance to AS and/or MQ. Both genes were amplified by PCR in 161 *Plasmodium falciparum* samples from the Peruvian Amazon Basin taken in the years 1999 (*n*=104) and 2006 (*n*=58). Length variation was assed in 6 microsatellite markers loci flanking *Pfmdr1*. Molecular analysis was done by sequencing the *Pfmdr1* SNP 86, 184, 1034, 1042 and 1246; and by sequencing *Pfatp6* gene coding region to identify novel mutations. *Pfmdr1* alleles N86, 184F and 1042D were fixed in the Amazon Basin. Alleles S1034C and D1246Y were negatively selected by MQ+AS. Genetic diversity around *Pfmdr1* in 1999 (Hz=0.3397±0.21) was lower than in 2006 (Hz=0.4343±0.26). Polymorphisms found in the *Pfatp6* gene in 1999 were L402V, S466N, C(tgc)1030C(tgt) and a G deletion in codon 884; in 2006 were A630S and V1168I. The simple mutant 884 genotype was selected by the MQ+AS treatment. Genotype 402/630/1168 was only present in 2006. Mutant alleles in *Pfmdr1* at codons 1034, 1042 and 1246 selected by CQ would benefit the MQ+AS treatment. The presence of the 1042D allele could render isolates sensible for MQ. Addition of the other mutations could increase this sensibility. Also, mutant alleles in *Pfatp6* at codons 630 and 1168 could be potential molecular markers for MQ or/ and AS resistance.

198

HITCHHIKING AND SELECTIVE SWEEPS OF *PLASMODIUM FALCIPARUM* SULFADOXINE AND PYRIMETHAMINE RESISTANT ALLELES IN CAMEROON

Andrea M. McCollum[1], Rachida Tahar[2], Leonardo K. Basco[2], Venkatachalam Udhayakumar[1], Ananias A. Escalante[3]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Unité de Recherche "Paludologie Afro-tropicale," Institut de Recherche pour le Développement (IRD) and Laboratoire de Recherche sur le Paludisme, Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale (OCEAC), Yaoundé, Cameroon, [3]Arizona State University, Tempe, AZ, United States

Drug treatment of *Plasmodium falciparum* infections has led to the selection for resistant mutant alleles. Sulfadoxine pyrimethamine (SP) resistance is encoded by a number of mutations in the genes dihydrofolate reductase (*dhfr*) and dihydropteroate synthetase (*dhps*). Here, we have genotyped mutations in *dhfr* and *dhps*, and have characterized microsatellite loci around *dhfr* on chromosome 4 and *dhps* on chromosome 8 as well as neutral markers on chromosomes 2 and 3 in 332 samples from Yaoundé, Cameroon. Our goals were to investigate the effects of selection on *dhfr* and *dhps* in this population, determine

the genetic relationships among *dhfr* and *dhps* alleles, and test the single origin hypothesis of highly pyrimethamine resistant alleles in a population from central Africa. Only 5% of the samples had wildtype *dhfr* or *dhps* alleles. This population shows strong linkage disequilibrium between the markers surrounding *dhfr* and *dhps* independently and little linkage within or between the neutral markers on chromosomes 2 and 3 - the result of strong selection on *dhfr* and *dhps*. The Cameroonian population shows skewed haplotype frequencies and a reduction of variation for mutant *dhfr* and *dhps* alleles, both are characteristics of selective sweeps occurring in this population. The previously reported Southeast Asian triple mutant *dhfr* haplotype is the most predominant in this sample set, but we also find additional independent, local haplotypes at low frequency. We also find multiple haplotypes for *dhps* mutant alleles; thus there have been multiple, independent originations of the mutant *dhfr* and *dhps* alleles in this population. This indicates that selection may act differently on *dhfr* and *dhps* within a population. These results yield support for the use of microsatellite markers to track resistant parasites in populations with a great amount of genetic diversity. In addition, this study demonstrates the signature of strong natural selection in a population with a great amount of recombination.

199

GENETIC ANALYSIS OF THE RETURN OF CHLOROQUINE SENSITIVE *PLASMODIUM FALCIPARUM* PARASITES TO LAMBARÉNÉ, GABON

Nicola Lehners[1], Pembe Mayengue[1], Julian Gabor[1], Matthias Dal-Bianco[1], Ghyslain Mombo Ngoma[2], Christian Supan[2], Bertrand Lell[2], Francine Ntoumi[1], Martin Grobusch[1], Peter Kremsner[1], **Frank Matthias**[1]

[1]Institute for Tropical Medicine, University of Tuebingen, Tuebingen, Germany, [2]Medical Research Unit, Albert Schweitzer Hospital, Lambaréné, Gabon

Chloroquine resistance is mediated by the K76T allele of the *Plasmodium falciparum* chloroquine resistance transporter gene (*pfcrt)* and is highly prevalent throughout Africa. Due to a genetic sweep the chromosomal haplotype surrounding the *pfcrt* locus is largely conserved in resistant parasites. Recently it has been observed that cessation of chloroquine use can lead to a return of sensitive parasites. The exact genetic mechanism of this phenomenon however remains unclear. Here we attempt to address this question via haplotype analysis in the setting of the formerly 100% resistant area of Lambaréné, Gabon, where chloroquine was removed from the national treatment guidelines in 2003. We screened parasite DNA from 90 samples obtained in 2005/06 and 54 samples obtained in 2007 for the sensitive *pfcrt* allele and found 1 sensitive isolate in 2005/6 and 3 in 2007. Sequence analysis revealed that all 4 sensitive isolates carried the same sensitive allele. Chromosomal haplotype analysis with microsatellite markers revealed two different sensitive haplotypes: one without similarities to the resistant haplotype and one that is identical to the resistant haplotype extending to approximately 20kb upstream of the *pfcrt* gene at which point the sequences start to diverge. This suggests that the sensitive allele is incrossing into the resistant population. In addition analysis of 145 resistant samples obtained over a time period from 1995 to 2007 revealed a decreasing prevalence of the dominant resistant haplotype from 79% in 1995-96 to 58% in 2005-2007. Removal of chloroquine from the national treatment guidelines in Gabon appears to have coincided with reintroduction of the sensitive *pfcrt* allele by immigration and incrossing.

200

HERITABILITY OF PARASITE CLEARANCE TIME FOLLOWING ARTEMISININ COMBINATION THERAPY

Tim Anderson[1], Shalini Nair[1], Ric Price[2], Marion Barends[3], Anchalee Jaidee[3], Francois Nosten[3]

[1]Southwest Foundation for Biomedical Research, San Antonio, TX, United States, [2]Menzies School of Health Research, Darwin, Australia, [3]Shoklo Malaria Research Unit, Mae Sot, Thailand

Malaria parasites in some SE Asian locations show slow clearance after treatment with Artemisinin Combination Therapy (ACT). This has lead to alarm about the evolution of resistant parasites. But are parasite genes really responsible? To answer this question we measured the heritability of parasite clearance time in parasites from the Thai-Burma border. We measured clearance time at 24 or 6 hr intervals in 185 single clone parasite infections following ACT treatment, and characterised parasites by genotyping 423 microsatellites markers distributed at ~50kb intervals through the genome. We found many clonally identical or closely related parasites in this population sample. We therefore asked whether such parasites tend to show similar clearance times, using methods analogous to those used in human twin and pedigree studies. While we found a wide range of clearance times, there was no significant impact of parasite genes on clearance time. In contrast, *in vitro* resistance to a variety of drugs showed strong heritability in the same parasite collection, demonstrating that we have adequate power to detect genetic effects with this study design. These results provide a cautionary tale about the dangers of using *in vivo* phenotypes for case-control association studies because these may be influenced by multiple factors. We conclude that variation in clearance times is not influenced by parasite genotype on the Thailand-Burma border. However, we caution that this conclusion may not hold in other locations, such as the Thailand/Cambodia border, where unusually slow clearance times have been reported.

201

CHARACTERISTICS OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN CHILDREN AGED 6-59 MONTHS IN THE KASSENA NANKANA DISTRICT OF NORTHERN GHANA

Daniel Tindanbil

Navrongo Health Research Center, Navrongo, Ghana

An effective malaria vaccine remains the most viable option to effectively fight malaria. Such a vaccine will possibly target children between the ages of 6 to 59 months since this is the group mostly affected by malaria. Uncomplicated malaria could be a potential trial endpoint. Proper identification and understanding of the seasonal variation of uncomplicated malaria cases in the Kassena Nankana District could also lead to improvement in diagnosis and early management of malaria cases in the district. This study therefore, describes age-specific levels of uncomplicated malaria as well as the seasonal variations of its infection within the Kassena Nankana District of northern Ghana. This study was conducted in the Kassena Nankana District of Northern Ghana, an impoverished rural area with hyper endemic transmission of malaria. Children between the ages of 6- 59 months presenting at a district hospital and four health centres within the district with clinical symptoms suggestive of uncomplicated or mild malaria were recruited into this study.1642 were enrolled. Analysis was done using stata 9.0. Age specific levels of infection and seasonality of transmission were calculated. 80% of study participants were between 6 and 24 months old.58% had auxiliary temperature of ≥ 37.5 while the remaining 42% reported history of fever within the last 24 hours preceding the interview. Other presenting symptoms were shaking chills or rigor, (45%), vomiting, (64%). 99.88% of study participants were diagnosed with *Plasmodium falciparum*.The remaining 0.12% of the cases were due to *P. malariae* infection. Most of the cases were recruited between the months of June and September, coinciding with the onset and intensity of rain fall. In conclusion, malaria prevention and treatment programmes will have to give special attention

to children between 6 and 24 months old since they are mostly affected by the disease in the Kassena Nankana District of northern Ghana.

202

PROSPECTS FOR MALARIA ELIMINATION IN MELANESIA

G. Dennis Shanks

Australian Army Malaria Institute, Brisbane, Australia

Since announcements by the Gates Foundation and the World Health Organization in October 2007, malaria elimination has again become a topic for the global public health agenda. This is more an aspiration than a target as current malaria control methods do not permit the elimination of malaria (local eradication) from areas with stable transmission. Malaria elimination has been previously attempted on small isolated Melanesian islands with some cases of success in Papua New Guinea, Solomon Islands and Vanuatu. Malaria transmission returned when new cases were introduced from outside the island. The southern most Vanuatu island of Aneityum showed that intensive vector control combined with mass drug treatment could eliminate malaria given a vigorous campaign to screen all island entrants for malaria parasites. The very high level of community participation on Aneityum was driven by specific economic incentives. Malaria control reducing clinical disease to very low levels is quite possible using insecticide treated bed nets. The problem is the elimination of even low-level transmission particularly of the more robust *Plasmodium vivax*. Use of long-acting 8-aminoquinolines such as tafenoquine may be effective in eliminating relapses from the liver but the use of 8-aminoquinolines including primaquine is limited by the presence of the human enzymopathy of glucose-6-phosphate dehydrogenase. One of the margins of malaria transmission runs through Melanesia and will be the focus of further efforts towards malaria control. Given the renewed interest in malaria elimination, it may be possible to push the malaria transmission border north through the other islands of Melanesia.

203

EPIDEMIOLOGY OF MALARIA IN FOREST AND PLAIN ECOTYPES OF NORTHERN ORISSA, INDIA

Surya K. Sharma

National Institute of Malaria Research, Rourkela, India

The state of Orissa in eastern India accounts for ~25% of malaria cases, ~40% of *Plasmodium falciparum* cases and ~25% of deaths attributed to malaria in the country, although it constitute only ~4% of total population of India. Therefore, studies were initiated in 2001 in Sundargarh district of Orissa to understand epidemiology of malaria transmission. The primary focus of the studies was to collect clinical, entomologic and epidemiologic indicators from the study site through longitudinal and cross-sectional surveys. There are 35 study villages (forest-23, plain-12) with a total population of 15847. The longitudinal epidemiological studies were conducted in two sets of villages in the forest and plain areas characterized by hyper and mesoendemic malaria situations respectively. In forest area, malaria transmission is perennial and *Plasmodium falciparum, P.vivax* and *P.malarie* accounts for 85.0, 14.0 and 1.0 percent of total malaria cases. Malaria transmission is also perennial in plain area villages but markedly low compared to forest villages. On average the number of *P. falciparum* cases per 1000 population per year in forest and plain villages was 280.0 and 21.5 respectively. The average parasite rate in forest and plain ecotypes was 17.4% and 1.5% respectively. In forest area, clinical malaria occurs more frequently in children aged 0-5 years and declines gradually with increasing age. Malaria vector *An. culicifacies* was the most predominant species in forest and plain area, whereas *An. fluviatilis* was restricted to only forest area. Studies on the host feeding behaviour of the vectors and entomological inoculation rate (EIR) in both the areas during different transmission seasons were also conducted. The EIR in the forest and plain area was 0.311 and 0.014 infective bites/person/night respectively. The study showed that villages in forest and plain areas separated by short geographical distances have distinct epidemiology of malaria transmission.

204

THE ROLE OF MALARIA PARASITAEMIA IN THE OCCURRENCE OF ANAEMIA IN FEBRILE ADULTS IN BUEA, CAMEROON

Ebako N. Takem[1], Eric A. Achidi[2], Peter M. Ndumbe[2]

[1]Ministry of Health, Buea, Cameroon, [2]University of Buea, Buea, Cameroon

Malaria is a major parasitic disease in developing countries. Anaemia is common in malaria but its role in adult patients has not been much studied. Our objective was to compare haemoglobin levels in patients with malaria parasitaemia to that of patients without malaria parasitaemia. A cross-sectional study was carried out from November 2007 to April 2008 in 4 health units in Buea, Cameroon. Patients who satisfied all of the following criteria were included in the study: fever (axillary temperature greater than or equal to 37.5 degrees centigrade) or history of fever, age 18-65 years, informed consent provided. Information on socio-demographic variables was collected using a questionnaire. Malaria parasitaemia status was determined using a Giemsa stained thick blood smear examined by microscopy. Haemoglobin levels were determined using the microhaematocrit technique. Data entry was done using EPIDATA version 3. Statistical analysis was performed using STATA version 8.2. The study population consisted of 156 adult patients with a mean age of 30.29 (SD=10.93) years and 59% of the patients were females. 31% of the patients had malaria parasitaemia and 15% had anaemia (haemoglobin<11g/dl). Multiple regression revealed that those with malaria parasitaemia had lower haemoglobin levels compared to those without malaria parasitaemia (coefficient b= - 0.8, 95% CI= -1.40, -0.21, p=0.009) after adjusting for age, sex, rural or urban residence, socioeconomic status, mean corpuscular volume and platelet count. In conclusion, in adult patients with fever or history of fever suggestive of malaria, those with malaria parasitaemia are more likely to have lower haemoglobin levels. Health interventions in adults based on reducing anaemia should be targeted towards controlling malaria.

205

PREDICTING GLOBAL FUND GRANT DISBURSEMENTS FOR PROCUREMENT OF ARTEMISININ-BASED COMBINATION THERAPIES

Justin M. Cohen, Inder Singh, Meg E. O'Brien

Clinton Foundation HIV/AIDS Initiative, Boston, MA, United States

An accurate forecast of global demand is essential to stabilize the market for artemisinin-based combination therapies (ACTs) and to ensure access to high-quality, life-saving medications at the lowest sustainable prices by avoiding underproduction and excessive overproduction, each of which can have negative consequences for the availability of affordable drugs. A robust forecast requires an understanding of the resources available to support procurement of these relatively expensive antimalarials, in particular from the Global Fund, at present the single largest source of ACT funding. Predictive regression models estimating the timing and rate of disbursements from the Global Fund to recipient countries for each malaria grant were derived using a repeated split-sample procedure intended to avoid overfitting. Predictions were compared against actual disbursements in a group of validation grants, and forecasts of ACT procurement extrapolated from disbursement predictions were evaluated against actual procurement in two sub-Saharan countries. Quarterly forecasts were correlated highly with actual smoothed disbursement rates (r=0.987, p<0.0001). Additionally, predicted ACT procurement, extrapolated from forecasted disbursements, was correlated strongly with actual ACT procurement supported by two grants from the Global Fund's first (r=0.945, p<0.0001) and fourth (r=0.938, p<0.0001) funding rounds. This analysis derived predictive regression models that successfully forecasted disbursement patterning for individual Global Fund malaria grants. These results indicate the utility of this approach for demand

forecasting of ACTs and, potentially, for other commodities procured using funding from the Global Fund. Further validation using data from other countries in different regions and environments will be necessary to confirm its generalizability.

206

GENOTYPING OF *PLASMODIUM VIVAX* ISOLATES FROM ARMENIA

Michela Menegon[1], Patrick Durand[2], Vladimir Davidyants[3], Giancarlo Majori[1], François Renaud[2], **Carlo Severini**[1]

[1]Istituto Superiore di Sanità, Rome, Italy, [2]Centre National de la Recherche Scientifique/Institut de Recherche pour le Developpement, Montpellier, France, [3]National Health Institut of Armenia, Yerevan, Armenia

Plasmodium vivax is the most widely distributed human parasite and the main cause of human malaria outside Africa. Recently, a research project funded by the European Commission (INCO Copernicus-2 project contract no. ICA2-CT-2000-10046) on the molecular epidemiology of *P. vivax* malaria has been implemented in three countries belonging to the Community of the Newly Independent States (NIS-Countries), i.e. Armenia, Azerbaijan and Uzbekistan. To investigate the genetic makeup of the *P. vivax* population in Armenia, a study was carried out in the endemic regions of Ararat and Yerevan. A total of thirteen *P. vivax* isolates were collected from patients attending the local health centres from July to October 2004. Genotyping analysis was carried out combining different available molecular tools, namely polymerase chain reaction (PCR) amplification and sequencing of a polymorphic *pvmsp-1* gene region as well as tandem repeat and microsatellite markers analysis. Plasmodial DNAs were extracted from infected blood samples spotted onto filter papers, using the QIAamp DNA blood Kit (QIAGEN), following the manufacturer's instructions. *Pvmsp-1* fragment amplification was performed as previously described. All PCR products were sent to MWG Biotech (Germany) for sequencing; subsequently, *pvmsp-1* sequences were compiled and analyzed at the ISS laboratory using the *DSGene* 1.5 computer program. Results from *pvmsp-1* amplicons sequencing were used to identify the representative MSP-1 types described so far, i.e. Belem and Sal-1 and recombinant types. Among the thirteen Armenian isolates analyzed, eight have been ascribed to Belem type and five to Sal-1 type. We have also analyzed eight tandem repeats and fourteen microsatellite loci. The number of identified alleles varies from one to seven. One allele of a TR locus and two alleles of two microsatellite loci were detected which had never been found in our previous studies. Thus, the *P. vivax* Armenian population shows noticeable genetic diversity when compared to the low endemic situation for malaria in this region.

207

THE TEMPORAL DYNAMICS OF *PLASMODIUM* DENSITY THROUGH THE SPOROGONIC CYCLE WITHIN *ANOPHELES* MOSQUITOES

Emma J. Dawes, Shijie Zhuang, Robert E. Sinden, María-Gloria Basáñez

Imperial College London, London, United Kingdom

In order to develop mathematical models that describe the sporogonic cycle of *Plasmodium* within the mosquito vector using a parasite density framework and evaluate the impact of transmission blocking strategies, it is important to determine the temporal dynamics of the various developmental stages within the mosquito and the effect of parasite density on such dynamics. A series of three experiments, in which cages of *Anopheles stephensi* mosquitoes were fed on blood infected with a range of *Plasmodium berghei* ookinete densities (from 50 to 2,000 per µl), were conducted to determine patterns of oocyst and salivary gland sporozoite abundance over time after infection and throughout the course of the entire sporogonic cycle. Every 24-48 hours after membrane feeding, samples of 20 live mosquitoes were dissected and the number of established oocysts and salivary gland sporozoites counted. Both the average and the raw counts for each time-point were analyzed. The results from these experiments were used to parameterize a compartmental model (comprising differential equations for ookinetes, oocysts, and sporozoites) and to quantify the rates of progression between the different *Plasmodium* developmental stages. Results indicate that the magnitude of these transition rates depends on parasite density with implications for our understanding of the impact of transmission blocking strategies on malaria transmission.

208

ASSESSMENT OF SEXUAL STAGE SPECIFIC IMMUNITY IN CHILDREN (0-15 YEARS OF AGE) IN ZAMBIA

Ralph E. LeBlanc[1], Tamaki Kobayashi[2], Phil Thuma[1], Nirbhay Kumar[1]

[1]Malaria Research Institute of the Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Plasmodium falciparum transmission reducing immunity has been demonstrated to correlate with immune responses to sexual stage specific antigens Pfs48/45 and Pfs230, depending upon the specific locale and age group studied. The development of transmission blocking immunity in subjects with natural exposure to malaria has been studied previously almost exclusively in adult populations and by cross sectional anlayses of subjects at one time point. Our study examined sexual stage specific immunity in children of various ages and over more than one transmission cycle. We conducted a prospective, longitudinal study of children [N=150 subjects] from age 0 to age 15 years in Zambia to determine the independent variables that may relate to the outcome of transmission reducing immunity as defined by the mosquito membrane feeding assay. Independent variables were age, sex, locale and history of maternal malaria infection in pregnancy. Malaria transmission is seasonable in Zambia and we documented a much lower rate of malaria infection by thick film during the dry season as compared to immediately following the rainy season. The relationship of specific immune responses to Pfs48/45 and Pfs230 as demonstrated by the subjects during the dry season as compared to immediately following the rainy season were analyzed to ascertain both the duration of transmission reducing immune responses as well as potential boosting of such responses by repeat exposure to malaria infection. Our analyses revealed a positive, statistically significant relationship between the age of the subjects and immune responses to Pfs48/45. In certain analyses, the locale or geographic location of the subject also emerged as a significant independent variable and further analysis of this finding is in progress. A more clear understanding of naturally induced transmission blocking immunity may be critical to the successful development of transmission blocking vaccines.

209

USE OF LOT QUALITY ASSURANCE SAMPLING (LQAS) TO MONITOR BEDNET DISTRIBUTION

Luis Benavente, Jose Luis Segura, Miguel Torrez

Medical Care Development International, Silver Spring, MD, United States

The Equatorial Guinea Malaria Control Initiative, EGMCI, distributed bednets in 144 housing/ distribution areas. Volunteers carried out a rapid household census; inventoried sleeping areas; and supplied the households with one LLIN per sleeping area. EGMCI did a concurrent rapid spot check using the LQAS methodology, in 19 households/census tract or lot.Indicators: A) Percent of households contacted and receiving at least one net. Threshold or decision rule:15 homes. B) Percentage of households with one bed-net per sleeping surface, threshold equal to 13 homes. Data collection: Households were selected using paper bits with sequential numbers. Sample size was proportion to the hamlet's population. 8037 households were visited, and 41,498 nets were distributed. For LQAS monitoring, 896 households from 61 lots (census tracts) were visited,later aggregated into five municipalities. Two municipalities failed for indicator

A and two failed for indicator B (one failed for both). Decision Rules were established only for process (ownership) indicators. Other projects may reduce sample size to 19 per municipality instead of 19 per census tract. A survey using 30 clusters with 10 households per cluster would have result in about a third of the number visited using the LQAS methodology described above. Such cluster sampling would not have identified failing census tracts for later quality control follow-up. Choosing the LQAS sample size is a compromise between the precision required to identify specific problem areas, the resources available to carry out the survey, and the larger costs of "mop up" that would be required in a larger area (necessary with smaller sample) as opposed to a smaller area (necessary with a larger sample)

210

SPONTANEOUS POSTPARTUM CLEARANCE OF *PLASMODIUM FALCIPARUM* IN BENINESE WOMEN

Valérie Briand[1], Julie Bottero[1], Achille Massougbodji[2], Michel Cot[1]

[1]Institut de Recherche pour le Développement, Paris, France, [2]Faculté des Sciences de la Santé, Cotonou, Benin

The effects and consequences of gestational malaria are well documented, still little is known about malaria in the immediate postpartum. A single study published in the 1980s demonstrated that women who were parasitaemic at delivery cleared their parasitaemia spontaneously within 48 hours postpartum. To confirm this phenomenon we investigated the spontaneous early postpartum evolution of malaria infections at delivery in Beninese women. Women were part of a large clinical trial which aimed to compare the efficacy of sulfadoxine-pyrimethamine and mefloquine for intermittent preventive treatment. Women who were infected with *Plasmodium falciparum* at delivery had a control of their parasitaemia in the early postpartum, as soon as the infection was detected. No antimalarial drugs were given unless women were symptomatic. Giemsa-stained thick blood smears were used to estimate parasite densities. Smears were recorded as negative if no parasite was detected after the examination of 200 microscope fields. 1601 women were recruited for the trial. Of them, 1346 (84%) had a peripheral thick blood smear at delivery. Thirty five (2.5%) women were infected with *P. falciparum* at delivery. For 17 (49%) of them, follow-up was not informative as they had received an antimalarial drug before being controlled. Eighteen women (51%) were not treated and they cleared their parasitaemia spontaneously. In these women, parasite clearance occurred within 5 days postpartum (2 days in median). At delivery, their median parasite density was 1659/mm^3 (range [93-85143]). Seventy-two percent (n=13) of them had a placental malaria infection simultaneously. They were primigravid in 50% of cases (versus 25% for the whole population of the trial). In conclusion, all women infected with *P. falciparum* at delivery who did not receive an antimalarial drug cleared their parasitaemia spontaneously within 5 days after delivery. This result supports the idea that, being a privileged site for the sequestration and multiplication of parasites, the placenta facilitates the persistence of parasitaemia during pregnancy, and its elimination induces a rapid clearance of parasites postpartum.

211

SINGLE-NUCLEOTIDE POLYMORPHISM IN *PLASMODIUM VIVAX* POPULATIONS FROM RURAL AMAZONIA

Pamela Orjuela-Sánchez, Mônica da Silva-Nunes, Natal Santos da Silva, Marcelo Urbano Ferreira

Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

Plasmodium vivax is the most prevalent human malaria parasite in Brazil. Understanding the genetic structure of *P. vivax* is essential to predict how fast phenotypes of interest, such as drug resistance, originate and spread in populations. Few genome markers are, however, available for the study of this species. Here we examined the levels of single-nucleotide polymorphisms (SNPs) diversity in 54 field isolates of *P. vivax,* collected in a well-characterized area in rural Brazilian Amazonia from March 2004 to May 2005. Two sets of SNPs were used: (a) 119 SNPs across 100 kb of chromosome 8 of *P. vivax* and (b) 11 SNPs at 2 loci (*pvmdr-1* and *pvcrt-o*) putatively associated with drug resistance in *P. vivax*. SNP typing was carried out by a competitive allele-specific polymerase chain reaction. Of the 119 SNPs analyzed, 34 (28.5%) were excluded because of poor or unspecific amplification. Of the 85 SNPs successfully typed, 39 were intergenic and 46 were located in open reading frames (ORFs). Of the 46 SNPs located in ORFs, 36 were synonymous and 10 were non-synonymous substitutions. Most SNPs (51) were monomorphic, and the probability of being monomorphic was significantly higher among SNPs located in ORFs (72%), than among those found in intergenic regions (46.1%) (P = 0.021, Fisher exact test). In the 54 parasite samples, we first analyzed the set of 85 SNPs at chromosome 8, revealing 40 different haplotypes among isolates. Only 4 haplotypes were shared by more than 1 isolate, and in 3 more instances of identical haplotypes were collected from the same subject with consecutive *P. vivax* infections, 2-3 months apart. These results suggest *P. vivax* relapses with genetically identical parasites. The analysis of SNPs in drug resistance associated genes *pvcrt-o* and *pvmdr1*, revealed that all 5 *pvcrt-o* SNPs tested were monomorphic (only wild-type found) in our samples population. The mutation Y976F of *pvmdr1*, previously described as associated with chloroquine (CQ) resistance, was found 3 of 48 samples analyzed and co-occurred with the mutation F1076L. Although *P. vivax* resistance to CQ remains uncharacterized in this area, we are currently evaluating the efficacy of CQ-primaquine regimen in local population. Further analyses of these results, combined with genotyping of additional parasites collected in the same area between August 2005 and August 2007, are expected to provide new insights into the temporal dynamics of haplotype diversity in *P. vivax*.

212

THE NET EFFECT OF NUMBERS: FACTORS ASSOCIATED WITH USE OF INSECTICIDE-TREATED NETS IN KENYA AFTER MASS DISTRIBUTION

Jimee Hwang[1], Paul Arguin[1], S. Patrick Kachur[1], Adam Wolkon[1], Joann Greenfield[2], Rebecca Kiptui[3], Laurence Slutsker[1], Willis Akhwale[3], Allen W. Hightower[4]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]World Health Organization, Nairobi, Kenya, [3]Division of Malaria Control, Nairobi, Kenya, [4]Centers for Disease Control and Prevention, Nairobi, Kenya

Mass distribution of large numbers of insecticide-treated nets (ITNs) is an important strategy to decrease the burden of malaria in endemic countries. In 2006, Kenya distributed 3.4 million ITNs to children <5 years in two mass campaigns. Our objective was to determine current ownership and use of ITNs in malarious areas of Kenya, with a focus on the factors associated with utilization. We conducted a nationwide Malaria Indicator Survey in June-July, 2007, during peak malaria transmission season using a personal digital assistant (PDA) based-questionnaire. Using probability proportional to size sampling, we selected 200 villages from 63 malarious districts in seven provinces. After mapping each village using Global Positioning System-equipped PDAs, we randomly selected 36 households per village. Univariate and multivariate logistic regression analyses were used to identify predictors for ITN use. Nationally, 62.5% of households (HH) (N=6854) owned one or more nets and 48.9% one or more ITNs, an increase from 21.8 and 5.9% pre-campaign, respectively. The night before the survey, 39.7% of children <5 (n=5864) and 41.7% of pregnant women (n=524) had slept under an ITN. Several demographic characteristics were independently associated with ITN use in HHs that own at least one ITN; however, in both univariate and multivariate analyses the strongest predictors of ITN use were age <5 years compared to those over 40 years (adjusted OR=1.64; 95% CI 1.40-1.91) and increasing number of ITNs in a household (adjusted OR= 2.16; 95% CI 2.00-2.33). Use for all persons, children <5 years, and pregnant women increased as the number of ITNs in the HH increased. Use for all persons living in a HH with only one ITN was 40.1%, two ITNs 61.1%, and three

or more ITNs 72.2%. Use for children <5 years living in a HH with only one ITN was 55.9%, two ITNs 73.6%, and three or more ITNs 76.4%. Use for pregnant women living in a HH with only one ITN was 64.8%, two ITNs 75.6%, and three or more ITNs 82.7%.This dose effect was significant for all three groups. The Kenya mass distribution campaigns dramatically increased ITN ownership and use, but only pregnant women living in a HH with three or more ITNs reached the Roll Back Malaria target level of 80%. Further efforts should aim to increase the number of ITNs distributed to each household and emphasize ITN education to increase utilization.

213

A COMPARISON OF THE IMPACT OF MALARIA CONTROL ACTIVITIES IN CAMBODIA MALARIA SURVEY BETWEEN 2004 AND 2007

Samphornarann Top

National Malaria Center, Phnom Penh, Cambodia

Malaria is one of the leading public health problems in Cambodia the 2007 Malaria Survey is to assess the performance and impact of malaria control activities in Cambodia in comparison with the results of the 2004 baseline. While the baseline survey had three domains only two of these will be included in the 2007 survey but add an extra risk zone beyond 2 kilometers from the forest to collect malaria-related data especially from the people who usually visit the forest. This change is based on the low malaria prevalence rate in domain 3, so it was decided to focus efforts and limited resources on the areas where data on malaria prevalence will be useful for action. The overall slide positively rate in higher risk regions, was 2.9%, the positive rate by *Plasmodium falciparum* was 1.6%, 0.9 % by *P. vivax* and 0.3% for mix infection. The infection was mostly high affected with the poorest people group if classified by socio-economic group and attacked to all age group. People more than 90% who know malaria transmission by mosquito bite when they went to forest and could be prevent by use of mosquito net. Up to more than 80%, from the poorest to less poor people in at risk was sleep under bed net. Around 70% households who recognize sign and symptom of malaria; 100% know well about danger sign of malaria and 93.3 % know where to go testing and treatment. But only 46.7% they were seeking treatment within 24 hours. The important finding of the survey is the similarity of epidemiological malaria data obtained from routine surveillance. It is around 40% reduction of malaria incidence among total population from year 2004 to 2007. People gained more knowledge on malaria prevention if compared to baseline result. In both survey results, It is significantly reduction from 4.4% (2004) to 2.9% (2007). There were significantly relationship between positive blood slide and risk areas, socio-economic and people who had fever. It is increased of percentage household sufficient net from 17.5 to 58.6. It is increased the percentage of awareness of anti-malaria drug among target population at risk from 47.3 to 72.The percentage of target groups who know that Malarine treatment is effective only if entire course is taken 10.3 in 2004 compared to 38.9 in 2007 survey. Based on result finding it showed that successfully for program implementation.

214

EFFECTS OF INSECTICIDE TREATED BEDNETS (ITNS) ON THE GENETIC DIVERSITY OF *PLASMODIUM FALCIPARUM* PARASITES IN A MALARIA HOLOENDEMIC AREA OF WESTERN KENYA

Wangeci Gatei[1], Simon Kariuki[2], William Hawley[3], Feiko ter Kuile[4], Dianne Terlouw[4], Penny Phillips-Howard[5], Bernard Nahlen[6], John Gimnig[3], Kim Lindblade[3], Edward Walker[7], John Williamson[3], Mary Hamel[5], Ananias Escalante[8], Laurence Slutsker[3], Ya Ping Shi[3]

[1]Centers for Disease Control, Division of Parasitic Diseases, Malaria Branch and Atlanta Research and Education Foundation, Atlanta, GA, United States, [2]Kenya Medical Research Institute, Center for Global Health Research, Kisumu, Kenya, [3]Centers for Disease Control and Prevention, Division of Parasitic Diseases, Malaria Branch, Atlanta, GA, United States, [4]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [5]Centers for Disease Control and Prevention, Division of Parasitic Diseases, Malaria Branch and Kenya Medical Research Institute, Center for Global Health Research, Atlanta, GA, United States, [6]President's Malaria Initiative, Washington, DC, United States, [7]Michigan State University, East Lansing, MI, United States, [8]Arizona State University, Tempe, AZ, United States

Use of insecticide treated bednets (ITNs) in sub-Saharan Africa has been shown to reduce malaria transmission by 70- 90%. However, there are information gaps on how this reduction in malaria transmission affects parasite population genetic structure. We assessed the impact of high coverage (>70% household ownership and use) with ITNs on *Plasmodium falciparum* parasite diversity using single copy multilocus microsatellite markers in an ITN trial site in Western Kenya. Seventy five parasite-positive blood samples collected from children less than 5 years of age in each period prior to (year 1998) and post (year 2001) introduction of ITNs (referred to as control and ITN periods respectively) were examined for length variation at the microsatellite loci. Analysis of the parasite population structure was based on the predominant allele present in each sample for each locus while the occurrence of multiple infections was quantified based on the identification of minor alleles. Preliminary data show high mean heterozygosities in the eight loci examined so far but no significant differences between the control (Mean 0.75, SE. 0.06) and ITN (Mean 0.71, SE. 0.07) periods. Based on average pairwise differences, significant differentiation was observed between the two parasite populations (Fst=0.0996; $p<0.05$) and significant genotypic differentiations at three out of eight loci in the two periods. Further intragenic analysis revealed significant differences in the variance in allele size between the control and ITN periods in one locus but most loci showed no differences. The proportion of samples with multiple alleles was higher than those with single alleles for both control (58%) and ITN (61%) periods. Further intraspecific analyses on multiple infections showed a significantly higher mean number of alleles in the control compared to the ITN periods in three loci. Strong and significant linkage disequilibrium (LD) was observed in the two parasite populations with the exception of one pair in the control period and two pairs of loci in the ITN period. Our preliminary data suggest the application of ITNs has effect on parasite diversity that will require further investigations and monitoring.

215

THE RELATIONSHIP BETWEEN MSP-1_{19} HAPLOTYPE PREVALENCE AND HUMORAL IMMUNITY DURING A TREATMENT-REINFECTION STUDY IN KENYA

Daniel J. Tisch[1], Chris Yohn[1], Peter Odada Sumba[2], John Vulule[2], James W. Kazura[1], Moormann M. Ann[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]Kenya Medical Research Institute, Kisumu, Kenya

The association between MSP-1_{19} haplotype-specific antibodies and infecting haplotypes was evaluated in a longitudinal treatment-reinfection study of 101 adults and 100 children in Kisumumu, Kenya. Haplotype distribution was estimated using a multiplex LDR-FMA with

Plasmodium falciparum specific primers. Haplotype-specific antibodies were quantified by ELISA and the functional quality of these antibodies was measured using invasion inhibitory antibodies for the 3D7 parasite strain. The distribution of haplotypes was compared cross-sectionally using chi-square tests and logistic regression to control for age, baseline parasite density, and baseline haplotype. Baseline infection was most commonly characterized by The FVO allele (54 of 126 infected). In this study population, 50% of adults and 25% of children had antibodies to 3D7 (p=0.0003) while 50% of adults and 25% of children had antibodies to FVO (p=0.1908). No significant differences were observed between baseline or reinfection haplotypes, and the reinfection haplotypes remained consistent with the baseline (i.e., circulating) distribution of haplotypes. 3D7-specific invasion inhibition was observed in 9 children and 1 adult (p=0.0089) and was not associated with time to reinfection (5.3 weeks versus 4.9 weeks for those with growth inhibition). Although none of the ten people positive for 3D7 invasion inhibition were positive for 3D7 haplotype at baseline, 3 of these were reinfected with 3D7 during the nine weeks of follow-up. No significant differences were observed between the measured functionality of antibodies and baseline or reinfection haplotype status. Existing and proposed malaria vaccines depend upon the development of humoral immunity to parasite strains including 3D7. However, this study did not detect an association with allele specific immunity to reinfection haplotypes in this naturally exposed population.

216

ACHIEVEMENTS IN MALARIA CONTROL IN ETHIOPIA: RESULTS FROM MALARIA INDICATOR SURVEY, 2007

Daddi Jima[1], Jimee Hwang[2], Asefaw Getachew[3], Hana Bilak[4], Estifanos Biru Shargie[3], Teshome Gebre[3], Gashu Fentie[3], Adam Wolkon[2], Scott Filler[2], Richard Reithinger[5], Paul M. Emerson[6], Tekola Endeshaw[3], Aryc W. Mosher[6], Frank O. Richards[6], Eskindir Tenaw[7], Ambachew M. Yohannes[8], Khoti Gausi[9], John Miller[4], Judith Robb-McCord[4], Richard Steketee[4], Patricia M. Graves[6], Zerihun Tadesse[1], Tedros Adhanom Ghebreyesus[10]

[1]Disease Prevention and Control Department, Ministry of Health, Addis Ababa, Ethiopia, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]The Carter Center, Addis Ababa, Ethiopia, [4]Malaria Control and Evaluation Partnership in Africa, PATH, Seattle, WA, United States, [5]United States Agency for International Development, Addis Ababa, Ethiopia, [6]The Carter Center, Atlanta, GA, United States, [7]Central Statistical Agency, Addis Ababa, Ethiopia, [8]World Health Organization, Addis Ababa, Ethiopia, [9]World Health Organization, Harare, Zimbabwe, [10]Minister of Health, Addis Ababa, Ethiopia

Malaria is a leading health problem in Ethiopia with 68% of its 77 million citizens at risk. The 2005 Demographic Health Survey (DHS) showed low levels of malaria prevention measures and treatment coverage. In 2006, the ministry of health launched a major initiative to scale up interventions, which included the distribution of nearly 20 million long-lasting insecticidal nets (LLIN), targeted indoor residual insecticide spraying, universal access to artemisinin combination therapy (ACT), and the training of 24,473 village-based health extension workers to enhance personal protection, health-seeking behavior, and case management. A cross-sectional national Malaria Indicator Survey was conducted during the major malaria transmission season (October-December, 2007) to assess intervention coverage and malaria and anemia prevalence. Using probability proportional to size sampling, 319 enumeration areas (EA) were selected. Each EA was mapped using a Global Positioning System and 25 households (HH) per EA were randomly selected for interview with a personal digital assistant based questionnaire. Analyses were weighted by selection probability. Nationally, HH ownership of nets increased from 6% in 2005 to 56% (N=7621). For HHs in areas of altitude <2000m, ownership was 69% and for the highly malarious regions (Afar, Benishangul-Gumuz, and Gambella) it ranged from 73-88%. Most (>95%) surveyed nets were LLINs. In 2005, net use by children under 5 years was 2%; it is now 35% in all HHs (n=5225), and 59% in HHs with a net (n=3206). In the twelve months preceding the survey, 14.2% of all HHs had been sprayed. Among children under 5 years, 22.3% reported fever in the two weeks prior to the survey. Of these, 15.4% sought medical attention within 24 hours of onset of fever and 9.5% took an antimalarial (of which 42.6% were ACTs). Although, malaria prevalence by microscopy for all ages was low, 0.7% (1.7% by Parascreen®), there was substantial regional variability ranging from 8.9% (20.7% by Parascreen®) in Benishangul-Gumuz to 0% by both tests in Addis Ababa. Anemia (Hg< 8.0 g/dL) was found in 5.5% of children under 5 years and was associated with malaria parasitemia (OR =3.9, 95% CI: 1.4-10.7). Compared to the 2005 DHS, the improvement in coverage with LLINs is remarkable. Continued investment into malaria control efforts are needed to maintain the encouraging progress towards achieving the Abuja targets and Roll Back Malaria goals in Ethiopia.

217

DEMOGRAPHIC PATTERNS OF *PLASMODIUM FALCIPARUM* INFECTION IN ANGOLA: USING A NATIONAL DEMOGRAPHIC SURVEY TO MEASURE PARASITEMIA

Shane Khan, Erin Eckert

Macro International Inc., Calverton, MD, United States

In Angola, the burden of malaria is alarmingly high, accounting for approximately 23 percent of under-5 child mortality. Until now, there has been no nationally-representative information on the patterns of *Plasmodium falciparum* infection among children. In 2006-07 a Malaria Indicator Survey (MIS) was conducted to measure parasitemia and coverage of key interventions (ITN, IRS). The MIS uses standard demographic sampling procedures to derive a nationally-representative, cross-sectional sample of households. In sample households, children aged 6-59 months (N=2497) were tested for *P. falciparum* using rapid diagnostic tests. The aim of this study is to present patterns of malaria infection in Angolan children, controlling for household and individual characteristics and protective behaviors. Results show that 19.5 % of children age 6-59 months is infected with malaria. Despite various controls, the odds of infection increase significantly with age, suggesting that protective behaviors are adopted while children are young, but lessen with time. Parasitemia varies considerably throughout Angola; children in rural areas are more than twice as likely as urban children to be infected (OR: 2.63, CI 1.68-4.12), and children in the mesoendemic region are significantly less likely to be infected (OR 0.58, CI 0.41- 0.84) than those in the hyperendemic regions. Parasitemia has a significant negative association with household wealth. Use of ITN the night before the survey is significantly associated with lower odds of parasitemia, though living in a household covered by IRS is not a significant predictor in these models. In further analysis, IRS alone has a significant protective effect in the mesoendemic unstable region where IRS coverage is higher (OR 0.16 CI 0.04 - 0.68) but the relationship disappears after controlling for other factors. There were no significant differences in parasitemia by sex of child and sex of head of household. The use of demographic surveys to examine program scale-up and impact is an important contribution to targeting of programs and examining impact.

218

RISK FACTORS ASSOCIATED WITH SEVERE MALARIA AMONG UNDER-FIVE CHILDREN IN SEVENTH-DAY ADVENTIST HOSPITAL, ILE-IFE, NIGERIA

Idowu O. Senbanjo[1], Philips Opreh[2]

[1]Lagos State University College of Medicine, Ikeja, Lagos, Nigeria, [2]Seventh-day Adventist Hospital, Ile-Ife, Nigeria

Most of the deaths from malaria are from the progression of uncomplicated malaria to severe malaria. The aim of this study was to determine the prevalence and risk factors associated with the development of severe malaria. This was a cross-sectional; hospital based prospective study of under-five children with malaria seen at the Seventh-day Adventist Hospital, Ile-Ife, Osun State, Nigeria during the

months of May through September 2005.One hundred and seventy six children fulfilled clinical and parasitological criteria for the diagnosis of malaria and were studied. Out of the 668 under-five children that were managed in the hospital during the study period, 176 (26.3%) children were diagnosed both clinically and parasitologically to have malaria. 116 (17.5%) children and 60 (9.0%) children had severe and uncomplicated malaria infections respectively. Of the seventeen variables examined, three remained independently and significantly associated with an increased risk for severe malaria. These were non-use of mosquito-bite preventive measures (RR = 3.502, 95% CI 1.279 - 9.586), low socio-economic class (RR =0.535, 95% CI 0.297 - 0.965) and high malaria parasite density (RR = 0.133, 95% CI 0.05 - 0.358). In conclusion, progress in stemming the burden of malaria depends on accurate knowledge and understanding of the epidemiology and control of the disease in the affected populations. The non-use of mosquito bite preventive measures might be as a result of ignorance and poverty. These factors should be considered in the design of sustainable and effective locally relevant strategies for the prevention of malaria.

219

A SIMULATION STUDY OF THE EPIDEMIOLOGICAL IMPACT OF MALARIA VACCINES

Melissa Penny, Nicolas Maire, Alain Studer, Allan Schapira, Tom Smith

Swiss Tropical Institute, Basel, Switzerland

A number of different malaria vaccine candidates against *Plasmodium falciparium* are currently in pre-clinical or clinical development. These vaccines vary in their characteristics, but it is unlikely any will provide long-lasting sterile immunity. There are many open questions concerning these, or future, vaccine candidates. There is need to investigate which transmission settings they will be appropriate for, which candidates should be considered for combining, what deployment strategies are advantageous and what minimal vaccine profile is needed before significant benefits are seen. We use a comprehensive simulation model of malaria to address such questions. Using our previously published stochastic simulation models we predict population effects of a range of malaria vaccines on malaria transmission, morbidity and mortality in realistic epidemiological and health system settings. The simulation model accounts for the dynamic effects of natural and vaccine induced immunity, for treatment of clinical episodes, and for births, ageing and deaths of the population. We simulate pre-erythrocytic vaccines (PEV), blood stage vaccines (BSV) and combinations with or without mosquito-stage transmission blocking vaccines (MSTBV) and consider a range of endemic malaria settings, deployment strategies, vaccine efficacies and half-life of protection. Our results indicate PEV have greatest benefits in low endemic settings but in some high transmission scenarios may lead to increased incidence of severe disease. BSV are predicted to be most useful in high transmission settings, and comparable to PEV in low transmission settings. A minimum half-life of 2-3 years is required for substantial epidemiological effects. Significant reduction in transmission is seen in some scenarios and herd immunity effects occur with moderately effective vaccines when deployed through mass campaigns targeting all ages, especially when combined with MSTBV. In conclusion, the results raise several issues for vaccine clinical development, in particular the appropriateness of vaccine types for different transmission settings; the need to assess transmission to the vector and duration of protection; and the importance of the choice of deployment strategy. To test the validity of our conclusions there is a need for further modeling.

220

CHARACTERIZATION OF THE KAMBILA, MALI COHORT FOR LONGITUDINAL STUDIES OF THE ACQUISITION AND MAINTENANCE OF NATURALLY-ACQUIRED MALARIA IMMUNITY

Kassoum Kayentao[1], Aissata Ongoiba[1], Boubacar Traore[1], Safiatou Doumbo[1], Didier Doumtabe[1], Younoussou Kone[1], Abdrahamane Traore[1], Greta Weiss[2], Seidina A. Diakite[1], Michael A. Krause[3], Seydou Doumbia[1], Aldiouma Guindo[1], Rick M. Fairhurst[4], Louis H. Miller[5], Susan K. Pierce[2], Peter D. Crompton[2], Ogobara K. Doumbo[1]

[1]Malaria Research and Training Center, Faculty of Medicine, Pharmacy and Dentistry, University of Bamako, Bamako, Mali, [2]National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Immunogenetics, Bethesda, MD, United States, [3]National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Malaria and Vector Research, Bethesda, MD, United States, [4]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [5]National Institutes of Health, National Institute of Allergy and Infectious Diseases, Malaria Vaccine Development Branch, Bethesda, MD, United States

Insight into the mechanisms responsible for naturally-acquired malaria immunity may facilitate malaria vaccine development. To this end we initiated a longitudinal study in the rural village of Kambila, Mali with these objectives: 1) characterize the malaria burden, 2) determine the demographic, genetic and environmental factors associated with malaria risk, and 3) test hypotheses related to the role of innate and adaptive immune responses in the acquisition of malaria immunity. Here we report on the first two objectives. One month before the 6 month malaria season, 225 individuals (aged 2-10 years and 18-25 years) were enrolled after random selection from an age-stratified census. From 495 clinic visits during the 1 year study period, 298 malaria episodes were diagnosed (T $\geq$37.5°C and asexual parasitemia >5000/µl). The average malaria incidence was 1.99, 0.98, and 0.08 among 2-7, 8-10, and 18-25 year olds, respectively ($p<0.001$). The median time to the first malaria episode, as measured in days since study enrollment, increased with age ($p=0.016$). Poisson regression showed that age (incidence rate ratio [IRR] 0.90 [95% CI, 0.85-0.95]; $p<0.001$), and HbAS (IRR 0.46 [95% CI, 0.27-0.79]; $p=0.005$) were significant predictors of decreased malaria incidence. Cox regression showed that age (hazard ratio [HR] 0.87 [95% CI, 0.80-0.94]; $p=0.001$), HbAS (HR 0.48 [95% CI, 0.26-0.91]; $p=0.024$), and asymptomatic *P. falciparum* parasitemia at enrollment (HR 0.35 [95% CI, 0.14-0.85]; $p=0.021$) were associated with decreased malaria risk. Factors that did not independently predict malaria risk by either measure were gender, ethnicity, weight, distance lived from clinic, self-reported bednet use, helminth infection, HbAC, G6PD deficiency, alpha thalassemia, and ABO blood group. For immunological studies, peripheral blood mononuclear cells and plasma were collected before, during and after the malaria season, and 14 days after the first malaria episode. By coupling rigorously characterized clinical and epidemiological data with recent advances in immunological methods, we aim to gain insights into the poorly understood process of naturally-acquired malaria immunity.

221

GENETIC DISRUPTION OF A MECHANOSENSITIVE ION CHANNEL IN *PLASMODIUM FALCIPARUM*

Kempaiah Rayavara, Sanjay A. Desai

Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

To transition from asexual blood forms in the human to reproductive forms in the mosquito, malaria parasites must undergo developmental changes referred to as gametocytogenesis. These changes are evidenced

by the formation of banana-shaped gametocytes in *P. falciparum*, the deadliest human malaria species. Although a number of gametocyte specific proteins have been identified by genome-wide expression arrays and protein sequencing, little is known about their biological roles. Here, we determined that one of these gametocyte specific proteins has a conserved small conductance mechanosensitive channel domain (MscS). In bacteria and other eukaryotes, ion channels containing this domain open in response to lateral tension in membranes and play diverse physiological roles including osmotic regulation, cell division and motility. This parasite protein (Pf-MscS) has five predicted transmembrane domains and is larger than most known MscS channels (predicted MW of 214 kDa). Quantitative RT-PCR confirmed diminishingly low expression in asexual blood stage forms that increases dramatically in gametocytes. Immunofluorescence assays using five specific antibodies raised in mice using both DNA vaccines and synthetic peptides reveal that the protein is expressed primarily at the gametocyte stage. To explore its physiological role, we have successfully disrupted the corresponding gene (*pf-mscs*) using double crossover homologous recombination. The phenotype of *pf-mscs* gene knockout parasites and approaches to targeting Pf-MscS for antimalarial drug or vaccine development will be discussed.

222

REFINING THE ANNOTATIONS OF THE *PLASMODIUM YOELII* MALARIA GENOME

Alfred Simkin, William Vincent, Gaya Hettiarachchi, Lindsey Howlett, Doug Armour, David Courtney, Caroline Hackett, Doug Hardesty, **Peter Blair**

Earlham College, Richmond, IN, United States

Malaria remains a global scourge to human life and existence and is responsible for up to 500 million cases and 3 million deaths annually. Therefore the need to establish and design novel malaria drug and vaccine strategies is crucial yet remains challenged, in part, due to the current status of malaria gene annotations. While the human malaria (*Plasmodium falciparum*) genome deserves the majority of attention, the accuracy of the rodent model (*P. yoelii*) genome is imperative for the traditional pipeline of drug/vaccine development and validity studies. Our research utilizes published large-scale datasets, notably Expressed Sequence Tags (ESTs), comparative genomics, and bioinformatic approaches to resolve and correct the current *P. yoelii* gene annotations. To date our research group has examined over 10% of the genes in the *P. yoelii* 17XNL genome (approximately 5,700 total predicted genes) and has successfully confirmed or corrected more than 100 genes, of which 60% required intron/exon boundary correction. This initial assessment justifies the need for annotation refinement. This presentation will offer both a description of our methodology and an update of results. Our data is currently available on the free online genomic resource, PlasmoDB (www.plasmodb.org), for dissemination to the greater malaria research community.

223

DISTRIBUTION AND GENETIC DIVERSITY OF *PLASMODIUM FALCIPARUM* ERYTHROCYTE BINDING ANTIGEN 175 (EBA 175) AND CLINICAL OUTCOME OF MALARIA IN CHILDREN IN THE KASSENA-NANKANA DISTRICT, GHANA

Beverly Egyir[1], Charles Brown[1], Langbon Bimi[2], Kwadwo Koram[1], Michael Wilson[1]

[1]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [2]University of Ghana, Legon, Accra, Ghana

The antigenic diversity of *Plasmodium falciparum* has been shown to influence the clinical manifestation of the malaria disease and also pose a major obstacle to antimalarial vaccine development. The search for an effective malaria vaccine has thus become a major public health challenge. The erythrocyte-binding antigen 175 (EBA-175) is a 175 kDaltons dimorphic antigen expressed on the merozoite of *P. falciparum*. The functions and potential effects of its dimorphism remain unclear. This study therefore investigated the relationship between this dimorphism and clinical outcome of malaria. A nested polymerase chain reaction (PCR) was used to genotype *P. falciparum* strains that exhibited this dimorphism in severe and mild malarial cases and healthy controls from the Kassena Nankana District (KND)of Ghana, an area which had been earmarked for future vaccine trials. A total of 299 samples were analysed, out of which 232 were positive with *P. falciparum* infections by PCR. The severe samples had a distribution of 44 (58.66%), 24 (32%) and 7(9.3%);the mild samples, 42 (55.3%), 29(38%), and 5(6.6%) ; and the healthy controls 59 (72%), 21(26%), and 13(16%) of the F, C and CF EBA-175 genotypes respectively. Chi-square analyses revealed that the mixed genotype (CF) was associated with severe malaria (OR=0.213, 95% CI: 0.042 - 1.076, p= 0.046) whilst the F genotype was significantly associated with reduction to the risk of having severe malaria (OR=0.907, 95% CI: 0.832-0.99, p=0.044). The results therefore suggest that EBA-175 genotypes are associated with the clinical outcome of malaria.

224

A PANEL OF GENETIC MARKERS SUGGESTS GENETIC RELATEDNESS AND EVOLUTIONARY HISTORIES OF *PLASMODIUM VIVAX* LINEAGES, OLD WORLD AND NEW WORLD

Surendra K. Prajapati[1], Hema Joshi[1], Simon Kang'a[2], Jane M. Carlton[2], Moshahid A. Rizvi[3], Aditya P. Dash[1]

[1]National Institute of Malaria Research, Delhi, India, [2]Department of Medical Parasitology, New York University Langone Medical Center, New York City, NY, United States, [3]Department of Biosciences, Jamia Millia Islamia University, Delhi, India

A previous study categorized *Plasmodium vivax* into two distinct lineages, Old World (Asia Pacific) and New World (South American), distinguishable by gene conversion in the S-type *SSU rRNA* gene, polymorphisms in an open reading frame (*ORF 470*) of the apicoplast genome, and mosquito transmission. With these features, New World *P. vivax* parasites were designated as a sub-species or separate species from Old World *P. vivax*. We have investigated this phenomenon using a panel of genetic markers to determine drug resistance potential, antigenic repertoires, selection pressure at vaccine candidate genes, genetic diversity and evolutionary histories of these two proposed lineages. Analysis of gene conversion in the S-type *SSU rRNA* from 354 *P. vivax* isolates from Indian sub continent revealed Old and New World sub types were equally prevalent in the Indian sub continent and suggest a lack of geographical sub-division between them. Polymorphism analysis of *dhfr* and *dhps* genes challenged the potential for development of resistance between Old and New World, suggesting that the two proposed lineages of *P. vivax* are equally capable of developing drug resistance. Antigen encoding genes from sexual and asexual stages revealed both low and high antigenic repertoires respectively that were found to be consistently similar among two lineages. Putatively neutral SNPs at ten housekeeping genes were found to be randomly distributed in the two lineages, and coalescence analysis of time to the most recent ancestor estimates of the age of *P. vivax* revealed ancient but overlapping evolutionary histories. Extensive genetic diversity was scored at ten minisatellites and eight microsatellites, and this extensive genetic diversity was found to be similar for both Old and New World *P. vivax* isolates. Phylogenetic trees derived from antigenic genes, housekeeping genes, microsatellites and minisatellites consistently revealed no separate clade of Old and New World parasites, providing no evidence for the hypothesis that New World *P. vivax* is a sub-species or separate species of *P. vivax.* Our panel of genetic markers consistently revealed similar patterns for drug resistance potential, antigenic repertoires, genetic diversity and evolutionary histories between Old and New World *P. vivax* parasites.

225

DIVERSITY IN THE LIGAND DOMAIN OF *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN: SPATIAL DISTRIBUTION ON 3-D STRUCTURE AND EVOLUTION

Taís N. Sousa[1], Eduardo M. Tarazona-Santos[2], Ana Paula Madureira[1], Paula Regina Kuser[3], Marcelo U. Ferreira[4], Luzia H. Carvalho[1], Cristiana F. Brito[1]

[1]René Rachou Institute/Oswaldo Cruz Foundation, Belo Horizonte, Brazil, [2]Department of General Biology/Federal University of Minas Gerais, Belo Horizonte, Brazil, [3]Laboratory of Bioinformatics/Brazilian Agricultural Research Corporation, Campinas, Brazil, [4]Department of Parasitology/ University of São Paulo, São Paulo, Brazil

Plasmodium vivax is the most prevalent human malaria species in Brazilian Amazon region. The establishing of the disease in the host depends on the merozoite invasion of new blood cells. The Duffy binding protein of *P. vivax* (PvDBP) is involved in the major pathway used by this parasite to invade human erythrocytes, making this protein an important anti-vivax vaccine candidate. The contribution of the host immune response to maintain the high diversity observed in the ligand domain of PvDBP (region II, $PvDBP_{II}$) has been controversial; traditional population genetic analysis which does not take in account the intralocus recombination may have contributed to this discrepancy. Here, we used the recently described omegaMap software which has the power to detect variation in both the selection parameter and the recombination rate across $PvDBP_{II}$ sequence. We identified regions along $PvDBP_{II}$ sequence subject to positive selection using 122 isolates of *P. vivax* from six different populations of the Brazilian Amazon area. High recombination rates and low linkage disequilibrium seems to be involved in generation and maintenance of $PvDBP_{II}$ haplotype diversity. Natural selection acts differentially across $PvDBP_{II}$. In fact, the gene presents neutrally evolving codons as well as codons under selective pressures. The most prominent result is the evidence for diversifying selection on one $PvDBP_{II}$ region containing known and putative T- and B-cell epitopes. By using a computational model, we were able to visualize the structural location of polymorphic residues in a 3-D model of $PvDBP_{II}$, demonstrating some of them in close relation with the erythrocyte binding residues. These results are expected under a model of immune escape in which polymorphic residues near the binding site elude binding of inhibitory antibodies, implying that positive selection acts preferentially on residues near the erythrocyte binding site. Altogether, our results suggest the importance of host immune system in the positive selection of mutations related to escape of the parasite.

226

GENETIC DIVERSITY OF THE MEROZOITE SURFACE PROTEIN-3α LOCUS IN *PLASMODIUM VIVAX* ISOLATES FROM SRI LANKA

Mette L. Schousboe[1], Rupika S. Rajakaruna[2], Gawrie N. Galappaththy[3], Priyanie H. Amerasinghe[4], Flemming Konradsen[1], Cally Roper[5], Ib C. Bygbjerg[1], Michael Alifrangis[1]

[1]Centre for Medical Parasitology, Copenhagen K, Denmark, [2]University of Peradeniya, Peradeniya, Sri Lanka, [3]Ministry of Health, Colombo, Sri Lanka, [4]International Water Management Institute, Andhra Pradesh, India, [5]London School of Hygiene and Tropical Medicine, London, United Kingdom

The *Plasmodium vivax* gene, PvMSP3-α is highly polymorphic, widely used to study the diversity of *P. vivax* populations and has been suggested as a potential vaccine candidate. The objectives of this study were to evaluate the usefulness of this locus as a marker of *P. vivax* diversity in *P. vivax* samples (n=381) collected between 2002 and 2006, from 9 districts across Sri Lanka and compare the results with other studies to describe and contribute to the global pattern of *P. vivax* diversity. The PCR analysis resulted in amplification of 313 *P. vivax* samples, and reveals three genotypes (A, B, and C), with A being the dominating genotype (83.4 %), while B and C were only found in 14.4 % and 2.3 % of the samples, respectively. A low number of mixed infections were also detected (3.6 %), and consisted mainly of A and B genotypes. Island-wide differences in occurrence were clearly evident. In particular, the occurrence of the C genotype differed between the Sri Lankan districts being totally absent in the Western districts while present in 6 % of the samples from the Northern districts. PCR-restriction fragment length polymorphism (RFLP) analyses extend the level of polymorphism observed and the Northern districts differ from the others being the most polymorphic region. A clear trend towards a lower frequency of the C genotype within the Sri Lankan *P. vivax* population was observed when compared with other published studies. Furthermore, the B genotype and the most prevalent A1 genotype found in this study were unique, while the C genotype was found to be globally observed. In contrast to other studies of PvMSP3-α diversity, this study estimates the level of PvMSP-3α polymorphism in a high number of samples collected from a geographically distinct area. Despite the restricted study region, a high level of polymorphism is seen, and three out of eleven genotypes are found to be unique. Our results highlight the usefulness of the PvMSP3-α locus as a tool for genotyping of *P. vivax* infections, but also suggest geographically differences in polymorphism which emphasis the need for further studies before it can be evaluated as a potential vaccine candidate.

227

GENETICS OF MALARIA IN THE PRÍNCIPE POPULATION

Maria de Jesus Trovoada[1], Lígia A. Gonçalves[1], Paulo Almeida[1], Roni Moya[2], Rita Neres[1], Rute Nascimento[1], João Costa[1], Isabel Marques[1], Artur Borja[3], António Coutinho[1], Carlos Penha-Gonçalves[1]

[1]Instituto Gulbenkian de Ciência, Oeiras, Portugal, [2]Centro de Histocompatibilidade do Sul, Lisboa, Portugal, [3]Hospital Dr Manuel Quaresma Dias da Graça, Príncipe, Sao Tome and Principe

Príncipe is a 115 Km^2 island with about 6.000 inhabitants and belongs to the São Tomé and Príncipe archipelago located in the Gulf of Guinea. This island is quasi-isolated from the geographical, epidemiological and population point of view, providing a unique scenario to study in malaria genetic epidemiology. We performed a population-based study to search for genetic factors that favour the malaria carrier status in the Príncipe population. In May 2005 we sampled 1390 volunteer donors, living in eleven separate communities of Príncipe. Parasitemia prevalence was 26.2% among asymptomatic individuals as determined by PCR in peripheral blood. We searched for correlations of the carrier status with 130 single nucleotide polymorphisms in 39 candidate genes that were previously related to malaria in man and mice as well as genetic markers of mitochondrial haplogroups. Single marker analysis, linkage disequilibrium maps and haplotype reconstruction and mitochondrial haplogroups stratification were used to identify the genetic variants that confer resistance to malaria in the Principe population. We present results relating to the two genes that showed the highest association to malaria carrier status: Endothelin receptor A, and Nitric Oxide Synthase 2A. The results revealed that the association of genetic polymorphism on these genes to the malaria carrier status is dependent on the genetic background factors representing the ethnic structure of the population.

228

IDENTIFICATION OF POTENTIAL TARGET GENES FOR MALARIA VACCINE DEVELOPMENT BY DIFFERENTIAL EXPRESSION PROFILING OF RADIATION-ATTENUATED *PLASMODIUM FALCIPARUM* SPOROZOITES

Benjamin U. Hoffman[1], Charlie Xiang[2], Michael Brownstein[2], **Anusha M. Gunasekera**[1]

[1]Sanaria, Inc, Rockville, MD, United States, [2]J. Craig Venter Institute, Rockville, MD, United States

Radiation attenuated *Plasmodium falciparum* sporozoites are the only immunogens that reproducibly protect >90% of human recipients against

malaria. Irradiation of sporozoites prevents their completion of the hepatocyte stage cycle; however, the mechanism behind this attenuation, the targets of protective immunity, as well as the effects of irradiation on sporozoite gene expression are not known. To gain insight into these unknowns, transcript profiles of irradiated (150 Gy) and non-irradiated *P. falciparum* sporozoites were compared. Twenty 70-nucleotide oligo-arrays representing the complete *P. falciparum* genome (7,256 parasite oligo probes) were separately hybridized with 3 different experimental preparations/batches of cDNA obtained from irradiated and non-irradiated sporozoites. From these twenty, 8 arrays were chosen for further analysis based on correlation analyses ($r^2 > 0.80$) between technical replicates. For each chosen array, differentially regulated genes were selected based on, 1) fold change (>1.5 in either direction) and 2) signal quality from each spot (rQuality>0.5). By this method, 60-80 up-regulated and 50-150 down-regulated genes were found in common among array samples within an experimental batch. Shared responders from the 3 experimental batches were then compared to determine which genes were consistently affected by irradiation in all 8 arrays. At this level of stringent selection, four genes were identified with high confidence; 3 genes of defined function (redox protein-PFC0166w, membrane protein-PFI1820w, and lipid metabolism protein-MAL8P1.37) and a hypothetical protein, were identified. We plan to test and elucidate the significance and role of these genes in conferring attenuation by first verifying their response to radiation using alternate methods.

229

CEREBRAL MALARIA GENETICS IN ANGOLAN CHILDREN

Maria R. Sambo[1], Maria Trovoada[1], Carla Benchimol[2], Lígia Deus[1], Vatúsia Quinhento[2], Paulo Almeida[1], Rute Vieira[1], Isabel Marques[1], António Coutinho[1], Carlos Penha-Gonçalves[1]

[1]Instituto Gulbenkian de Ciência, Oeiras, Portugal, [2]Hospital Pediátrico David Bernardino, Luanda, Angola

Several association studies have been conducted in different African populations to examine the role of host genetics in severe malaria. Most often, the candidate gene approach has been followed, as part of case-healthy control studies. We design a case-control study to search for genetic factors that specifically confers increased risk or protection against cerebral malaria but not to other clinical forms of *Plasmodium falciparum* infection. This study is being carried out in Hospital Pediátrico David Bernardino, in Luanda (Angola), a mesoendemic malaria area with stable transmission. Our sample includes a total of 714 children aged from 6 months to 13 years. Cases were 130 children with cerebral malaria and controls were ascertained in three groups: 153 children with other forms of complicated malaria (severe malaria anaemia and hyperparasitaemia), 142 with uncomplicated malaria and 319 uninfected children. Candidate genes were chosen for association analysis on basis of previously shown relevance for the pathogenesis of severe malaria in humans and in animal models. We genotyped all the children for 238 SNPs in the following genes: CD36, HMOX1, TLR9, TLR4, NOS2A, HBB, TGFβ1, TGFβ2, ICAM1, TNF, GPX1, DARC, PKLR, GYPA, G6PD, EDNRA, CCR5, CCR2, CR1, FCGR2B, MBL2, CD40LG, IL10RB, PECAM1 and HP. Preliminary results show that markers in HBB, G6PD, CD36, NOS2A, TGFβ2, GYPA, HMOX1 and EDNRA genes are significantly associated with cerebral malaria when compared with the three different control groups. Haplotype reconstruction analysis identified in some genes haplotypes governing association to cerebral malaria. These data are compared with published results from other African countries. This study represents the first data concerning malaria host genetics in the Angolan population.

230

SEQUENCE DIVERSITY OF THE *MSP2* GENE (MEROZOITE SURFACE PROTEIN 2) IN THE *PLASMODIUM FALCIPARUM* COLLECTION AT THE MR4/ATCC BIORESOURCE CENTER

Thavamani Rajapandi, Linda Amoah, Timothy Stedman

MR[4]/American Type Culture Collection, Manassas, VA, United States

The Malaria Research and Reference Reagent Resource Center (MR4) provides malaria research-related reagents to the international malaria research community. As part of our authentication efforts, sequence diversity profiles of the *msp2* gene of *Plasmodium falciparum* isolates at the MR4 collection were generated. *In vitro* culture-adapted and geographically diverse *P. falciparum* parasite isolates were tested for the diversity of the *msp2* gene by various approaches. We found that the amplification fragment length polymorphisms (AFLPs), the PCR-restriction fragment length polymorphisms (RFLPs), and the nucleotide sequence diversity (NSD) are varied and diverse among the culture-adapted *P. falciparum* isolates. We identified 50% of the isolates at the MR4 collection as belonging to a single msp2 group, which is similar to the genome sequenced 3D7 isolate, and we call this group 3D7 -type *msp2* gene (3D7T-msp-2). A second unique group comprised of about 40% of the isolates tested is named as FC27 -type *msp2* gene (FC27T-msp-2), and the remaining 10% of the isolates tested are highly diverse and do not fall into either the 3D7-type *msp2* or the FC27-type *msp2*. We therefore refer to this third family as the variable -type *msp2* gene (VT-msp-2) family. Oligonucleotide primer sets were developed for the allele-specific amplification and identification of either 3D7T-*msp2* or FC27T-*msp2* genes. In addition, a multiplex PCR-based RFLP is also being developed to uniquely identify the VT-*msp2* genes. These methods are also being used to specifically differentiate the mixed infections and the recrudescent isolates that arose during anti-malarial drug efficacy studies. The results will be discussed for the evaluation and the adaptation of these techniques for the unique identification of *P. falciparum* parasite isolates from clinical samples.

231

INSECTICIDE SUSCEPTIBILITY LEVEL OF *AEDES AEGYPTI* (LINN.) FROM MUNICIPALITY OF CABUYAO AND CITY OF MANILA, PHILIPPINES

Wilfredo E. Aure

Research Institute for Tropical Medicine, Muntinlupa City, Philippines

The insecticide susceptibility of *Aedes aegypti* adults and larvae from 3 study sites in Cabuyao and 3 study sites in Manila, Philippines were investigated using World Health Organization standard procedures. All the field strains tested were F2 produced from ovitrap-collected eggs. Bora-bora was used as the susceptible control strain in all assays. All the field-strain larvae were susceptible to temephos (LC50 =0.0018 to 0.0048 mg/li., LC95= 0.0023 to 0.0065 mg/li.). Using the discriminating dose, all the adult field strains from Manila were resistant to permethrin, and cyfluthrin (<80 % mortality). Resistance to deltamethrin (73% mortality) was observed only in 1 site barangay (Bgy. 184). For the study sites in Cabuyao, field strains were resistant to permethrin (< 80% mortality) in two sites (Bgy. Diezmo and Bgy. Pittland) and resistant to cyfluthrin (73% mortality) in one site (Bgy. Pittland). Deltamethrin susceptibility was found in Bgy. Mamatid (99% mortality) and incipient resistance in Bgy. Diezmo (83% mortality) and Bgy Pittland (91% mortality). All colony and field strains were susceptible to malathion (100% mortality) but were resistant to DDT (1-74% mortality). Continuous resistance monitoring should be conducted regularly in epidemic prone areas, to identify the efficacy of compounds; well-managed rotations of effective insecticides are recommended. In addition to the use of effective insecticides, public education and good insecticide dispersal practices (such as proper application at the right target sites, spraying at the proper time of day, and calibrating equipment properly) using pre- and post-spray surveillance is necessary. Such information will provide rational consideration of alternatives to

adulticides. Greater attention should be paid to source reduction and environmental sanitation, to decrease reliance on insecticides and reduce selection pressure on already resistant populations.

232

IDENTIFICATION OF METABOLIC INSECTICIDE RESISTANCE GENES IN *AEDES AEGYPTI* FROM MARTINIQUE (FRENCH WEST INDIES): FROM GENOTYPES TO PHENOTYPES

Sebastien Marcombe[1], Rodolphe Poupardin[2], Frédéric Darriet[1], Clare Strode[3], André Yébakima[4], Hilary Ranson[3], Jean-Philippe David[2], Vincent Corbel[1]

[1]Institut de Recherche pour le Developpement, UR[016], Laboratoire de Lutte contre les Insectes Nuisibles, Montpellier, France, [2]Laboratoire d'Ecologie Alpine (LECA) UMR CNRS-Université [5553], Equipe «Perturbations Environnementales et Xénobiotiques», Grenoble, France, [3]Vector Group, Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [4]Centre de Démoustication/Conseil Général de la Martinique, Fort de France, Martinique

Since more than 10 years, *Aedes aegypti* is responsible for severe dengue outbreaks in the Caribbean and particularly in Martinique (French West Indies). Organophosphates and pyrethroids were used for decades for mosquito control which contributed to increase the pressure of selection on resistance genes in field mosquito populations. Larval and adult bioassays carried out on a field-caught mosquito population (Vauclin) showed high levels of resistance to temephos (x175) and deltamethrin (x68). No insensitive acetylcholinesterase was detected in Vauclin strain whereas high Kdr-allelic frequency (0.86) was identified at 106 position (Iso to Leu). Biochemical assays showed higher oxidase, GST and esterase activities in the resistant strain compared to the susceptible strain in both larvae and adults. The use of classical synergists (DMC, DEF and PBO) confirmed the role of detoxification enzymes in insecticide resistance, in addition to the kdr mutation. Analysis of the expression of detoxification genes at both larval and adult stages using the 'Aedes detox Chip' allowed to identify several potential genes responsible for resistance. Fifteen probes were over-expressed in larvae of the resistant strain including 13 P450s (CYP4, CYP6 and CYP9 families), 1 GSTs and 2 CCEs (CCEae2C and CCEunk7o). Eleven probes were over-expressed in adults including 8 P450s (CYP9 and CYP6 families) and 2 CCEs (CCEae3a and CCEae4b). Interestingly, 4 CYP genes were over expressed at both life stages (CYP9J22v1, CYP9J22v2, CYP6Z6 and CYP6M6). A better understanding of metabolic resistance genes underpinning the resistant phenotypes is essential to implement more effective and sustainable dengue vector control strategies.

233

UNEXPEXTED NEUROTOXIC EFFECTS OF THE REPELLENT DEET OCCUR THROUGH AN INHIBITION OF ACETYLCHOLINESTERASE ACTIVITY IN INSECT AND MAMMAL NERVOUS SYSTEM

Vincent Corbel[1], Maria Stankiewicz[2], Cédric Pennetier[1], Didier Fournier[3], Jordi Molgo[4], Jure Stojan[5], Jean Marc Hougard[6], Bruno Lapied[7]

[1]Institut de Recherche pour le Developpement, UR[016], Montpellier, France, [2]University of N. Copernicus, Torun, Poland, [3]University of Paul Sabatier, Toulouse, France, [4]CNRS, Gif sur Yvette, France, [5]Institute of Biochemistry, Ljubljana, Slovenia, [6]Institut de Recherche pour le Developpement, UR[016], Cotonou, Benin, [7]University of Angers, Angers, France

The repellent N,N-diethyl-m-toluamide or DEET remains the gold standard for insect repellents. Unfortunately, the mode of action and toxicity of this repellent remains largely unknown. Here, were reported the effects of DEET on the insect and mammal nervous system using a complementary approach including toxicology performed on insect *in vivo*, electrophysiology, pharmacology and biochemistry. The results showed that DEET is not simply a behaviour-modifying chemical but also exert insecticidal effect on *in vivo* insects. External application of DEET on the cercal-afferent giant-interneuron synapses in the terminal abdominal ganglion of the American cockroach showed that DEET (from 0.5 to 1 µM and in presence of 1 µM atropine) increased drastically both amplitude and duration of the Excitatory Postsynaptic Potentials (EPSP). Additional electrophysiological recordings performed on isolated mouse phrenic-hemidiaphragm muscles showed that DEET (500 µM) is able to prolong about 3 fold the decay time constant of both synaptic potentials and currents. The *in vitro* effect of DEET on the activity of two purified AChE, one from an insect (Drosophila melanogaster) and the other from humans (AChE and BChE) confirmed that DEET (from 1 to 10 mM) inhibits the hydrolysis of ACh by AChEs and to diminish AChE carbamoylation rate by propoxur. These results then indicate that DEET is a competitive inhibitor for AChE. These findings have important implications for DEET usage and provide new material potentially explaining one cause underlying the gulf war sickness syndrome.

234

MONITORING THE OPERATIONAL IMPACT OF INSECTICIDE USAGE FOR MALARIA CONTROL ON *ANOPHELES FUNESTUS* FROM MOZAMBIQUE

Michael Coleman[1], Sonia Casimiro[2], Janet Hemingway[3], Brian Sharp[1]

[1]MRC, Durban, Durban, South Africa, [2]National Institute of Health, Maputo, Mozambique, [3]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Indoor residual spraying (IRS) has again become popular for malaria control in Africa. DDT was re-introduced into Mozambique's IRS programme in 2005 and is increasingly becoming the main insecticide used for malaria vector control in Mozambique. The selection of DDT as the insecticide of choice in Mozambique is evidence-based, taking account of the susceptibility of *Anopheles funestus* and *An. arabiensis* to all available insecticide choices, as well as operational costs of spraying. Sentinel sites were monitored for insecticide resistance using WHO bioassays and biochemical assays. Assays were conducted on 1-3 day old F1 offspring of field collected adult caught females to determine levels of insecticide resistance and gene resistance frequency in the malaria vector population. Previously lambda cyhalothrin had replaced DDT in Mozambique in 1993. However, resistance appeared quickly to this insecticide and, in 2000, the pyrethroid was phased out and the carbamate bendiocarb introduced. Low level resistance was detected by biochemical assay to bendiocarb in 1999 in both *An. funestus* and *Anopheles arabiensis*, although this was not evident in WHO bioassays of the same population. In surveys conducted between 2002 and 2006, the levels of bendiocarb resistance detected in *An. funestus,* populations using WHO bioassays increased. Probably due to elevated levels of Acetylcholinesterase levels found in the same populations. Pyrethroid resistance in populations, linked to elevated levels of monooxygenase activity, decreases with the reduction of pyrethroids for control. This process of monitoring resistance has been incorporated into an integrated malaria decision support aiding malaria control programmes to make informed decisions and policy.

235

BIOCHEMICAL AND KNOCKDOWN RESISTANCE OF *ANOPHELES GAMBIAE* TO PERMETHRIN AND DELTAMETHRIN (PYRETHROIDS) AT KPONE ON SEA IN THE GREATER ACCRA REGION OF GHANA

Kwadwo K. Frempong[1], Isabella Quakyi[2], Sulley K. Ben-Mahmoud[3], Irene Offei Owusu[1], Maxwell A. Appawu[1], Daniel Boakye[1]

[1]Noguchi Memorial Institute For Medical Research, Accra, Ghana, [2]School of Public Health, University of Ghana, Accra, Ghana, [3]African Regional Postgraduate Programme in Insect Science (ARPPIS), University of Ghana, Accra, Ghana

Anopheles gambiae is the predominant mosquito species in Ghana and has been reported to confer resistance to pyrethroids. This resistance is linked to kdr gene which affects the sodium channel gate in the nervous system. Mixed function oxidase, esterases and glutathione S transferase have also been reported to confer resistance to pyrethroids through insecticide detoxification. It is not well known whether resistant mosquitoes exhibit both or one mechanism at a time and why some resistant mosquitoes do not possess the kdr gene. Adult *An. gambiae* mosquitoes were collected from four sectors at Kpone using human landing catches. W.H.O susceptibility test for permethrin (0.75%) and deltamethrin (0.05%) were conducted for five replicates each using twenty F1 females of the adult mosquitoes. PCR was used to test for presence of kdr gene, species identification and molecular forms using one leg of each mosquito. Enzyme activities were tested on the same set of mosquitoes for mixed function oxidase, esterases and glutathione S Transferase using ELISA. Questionnaires on insecticide use were administered to 300 adults residing in the study area. Exposure to permethrin recorded 52% mortality (n=100) and that of deltamethrin recorded 83% mortality (n=100). Out of 180 samples tested using PCR, 87.8% possessed the kdr gene whiles 10% did not. Hybrids recorded 2.2%. For species identification, 94% were identified as *An. gambiae s.s* whiles 6% as *An. arabiensis.* Out of 169 *An. gambiae s.s*, 87% were M form and 13% S form. Enzyme activity showed that out of 180 samples, 26 % exhibited high activity for oxidase, 21% for glutathione S transferase and 24% for esterase compared to Susceptibles. Samples having the kdr gene and exhibiting high enzyme activity were 32%. Results showed that presence of kdr gene is more associated with resistance. Almost the same set of mosquitoes exhibited a high enzyme activity for the three enzymes tested. Few more samples will be tested to show the true picture of this study.

236

EFFECT OF REPEATED APPLICATION OF BIOLOGICAL LARVICIDES ON MALARIA TRANSMISSION IN CENTRAL CÔTE D'IVOIRE

Tchicaya E. Stephane[1], Utzinger Juerg[2], Keiser Jennifer[2], Tanner Marcel[2]

[1]Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, Abidjan, Cote d'Ivoire, [2]Swiss Tropical Institute, Basel, Switzerland

There is growing political and financial commitment to eradicate malaria, and hence integrated control approaches, including biological larviciding, deserve attention. The purpose of this study was to investigate whether repeated application of *Bacillus thuringiensis* (Bti) and *B. sphaericus* (Bs) have an effect on malaria transmission. Larvae collection surveys conducted during 7 months showed the effectiveness of Bti and Bs. After the 8th treatment, *Anopheles* larvae were absent from the breeding sites. *Culex* larvae decreased after the 3rd treatment. Adult mosquitoes were captured by 56 man-nights in 2006 (February, May, August, and November), inside and outside households during two consecutive days. From a total of 2361 mosquitoes captured, 59.5% belonged to *Anopheles* genus. *An. funestus* s.l. was the most abundant, accounting for 82% of total Anophelines caught, followed by *An. gambiae* s.l. (17.2%). Both species were present all year long, with marked seasonal fluctuations. Peak abundance was observed during the rainy seasons, while, lowest biting rates were observed during the dry seasons. The comparison of entomological transmission parameters recorded, with data from 2005, showed that larvicide treatments permitted a significant decrease of *An. funestus* (5.1 bites/person/night; $P < 0.001$) and *An. gambiae* (18.7 bites/person/night; $P < 0.001$) biting rates. The infestation rate was stable for both species, with a much higher rate observed for *An. gambiae* (15.1%) when compared to *An. funestus* (2.1%). The annual entomological inoculation rate (EIR) for *An. gambiae* (281 infective bites/person/night; $P = 0.088$) was similar in 2005 and 2006, while the annual EIR of An. funestus (142 infectives bites/person/night; $P = 0.005$) has been drastically reduced. The routine application of larvicides in mosquitoes breading sites decreased the number of breeding sites containing *Anopheles* and *Culex* larvae, which could have favoured the significant reduction of *An. funestus* and *An. gambiae* biting rates, and a drastic decrease of *An. funestus* entomological inoculation rate.

237

WIDE PYRETHROIDS AND DDT RESSTANCE IN *ANOPHELES GAMBIAE* SENSU LATO SAMPLES FROM CAMEROON

Hamadou N. Ndjemai

National Malaria Control Program, Yaoundé, Cameroon

Insecticides are the cornerstone of most vector control strategies in sub-Saharan Africa. Accordingly, the susceptibility of *Anopheles gambiae* sensu lato, the major vector of malaria in Cameroon to six insecticides, was assessed in a wide range of biogeographic settings to determine how resistance is distributed and enable informed selection of insecticides for vector control programmes. Two to three days old unfed female mosquitoes were exposed during one hour to an organochlorine (DDT), a carbamate (Bendiocarb), an organophosphate (Malathion), and three pyrethroids (Deltamethrin, λ-Cyhalothrin and Permethrin) using the World Health Organisation bioassay test kits. The knockdown times of 50% and 95% of mosquitoes and mortality 24 hours post exposure were recorded and compared to the Anopheles gambiae Kisumu reference susceptible strain. Mortality was above 95% for DDT and pyrethroids in the Northern Anopheles arabiensis populations. Conversely, the Southern Anopheles gambiae sensu stricto populations recorded less than 90% mortality. Similarly, knockdown percentages were higher in Anopheles arabiensis populations than in *An. gambiae* sensu stricto. Moreover, pyrethroids resistance was higher in *An. gambiae* S molecular form than in the M form. However, no resistance to Bendiocarb and Malathion was found. This study indicated a wider distribution of DDT and pyrethroids resistance in *An. gambiae* sensu stricto than *An. arabiensis* populations. Interventions involving careful use of organophosphates and carbamates should thus be included in malaria prevention programmes in Cameroon.

238

EVALUATION OF MOSQUITO DENSOVIRUSES FOR CONTROLLING *AEDES AEGYPTI* (DIPTERA: CULICIDAE): VARIATION IN EFFICIENCY DUE TO VIRUS STRAIN AND GEOGRAPHIC ORIGIN OF MOSQUITOES

Supanee Hirunkanokpun

Mahidol University, Bangkok, Thailand

Four mosquito densovirus strains were assayed for mortality and infectivity against *Aedes aegypti* larvae from different geographic regions. The viral titers were quantified by real-time PCR using TaqMan technology. First instar larvae were exposed to the same titer of each densovirus strain for 48 hours. All strains of densoviruses exhibited larvicidal activity and caused more than 80% mortality and infectivity in the three mosquito strains. *Aal*DNV-exposed larvae had the highest mortality rate. The mean time to death of *Aal*DNV-exposed larvae was shorter than other DNVs-exposed larvae. We can conclude that different densovirus strains exhibit some variations in their pathogenicity to different populations of *Ae. aegypti* mosquitoes. A few mosquitoes from Chachoengsao and Bangkok

exposed to *Ae*DNV and *ATh*DNV survived to the adult stage to lay eggs and showed 22% to 50% vertical transmission in the F1 generation. Phylogenetic analysis of four densovirus strains indicated that mosquito densoviruses are separated into two distinct clades.

239

SIMALIKALACTONE D IS RESPONSIBLE FOR THE LARVICIDAL PROPERTIES OF *QUASSIA AFRICANA*

Woquan Sama, Edith O. Ajaiyeoba

University of Ibadan, Ibadan, Nigeria

Recently, botanical and microbial insecticides have been increasingly used for mosquito control because of their efficacy and documented non-toxic effects on non-target organisms. The discovery of new insecticides is imperative because of the development of resistance by the mosquitoes to the readily available insecticides. The aim of this study was to isolate and characterize compounds from *Quassia africana* that are toxic to *Anopheles gambiae*. The crude methanol extract leaf, stem and roots of *Q. africana* was tested against forth instar larvae of *An. gambiae*, under laboratory condition. The root extract was partitioned into hexane, chloroform and ethylacetate. All these extracts were screened for larvicidal properties. The most active extract was subjected to column chromatography and fractions obtained were screened for larvicidal properties. The fraction with the highest bioactivity was subjected to repeated column chromatography and the isolated compound was evaluated for its potential toxicity to larvae of *An. gambiae*. All the experiments were accompanied by a control series. The structure of the active compound was elucidated using spectroscopic techniques. The root extract showed very strong larvicidal activity with LD_{50} value of 75 µg/mL. Partitioning of the root extract into solvents of different polarities and screening for toxicity on *An. gambiae* larvae revealed that the larvae were more susceptible to the chloroform soluble fraction. Further bioactivity-guided separation of the chloroform fraction of the root extract led to the identification and isolation of a simalikalactone D as the larvicidal compound in *Q. africana* with LD_{50} value of 2 µg/mL. The larvicidal properties of *Q. africana* and simalikalactone D are reported for the first time. Results from this study suggest that in addition to other medicinal properties of *Q. africana*, it may serve as a source for the development of vector control agents for malaria.

240

REPELLENCY FROM ESSENTIAL OILS AGAINST THE BITES OF *LUTZOMYIA MIGONEI* (DIPTERA: PSYCHODIDAE) *LEISHMANIA* VECTOR

Elsa Nieves[1], Janett Fernández[1], José Lia[2], Maritza Rondon[1]

[1]University Of The Los Andes, Merida, Venezuela, [2]Natural Products Laboratory, Botanical Garden, Unellez, Barinas, Venezuela

The present study evaluated the repellent effect of essential oils extracted from 8 species of plants against the bite from *Lutzomyia migonei*, vector of *Leishmania,* on humans using the ¨standard cage test¨ in laboratory conditions. The essential oils were extracted by hydrodistillation, from *Hyptis suaveolens* (mastranto) Poit; *Pimenta racemosa* (bay-ruum) (P. Mill) JW More; *Piper marginatum* (anise) Jacq; *Monticalia imbricatifolia* Schults; *Pseudognaphalium caruleocamum* (vira-vira) A. Steyermark Anderberg; *Espeletia shultzii* (frailejon) Weddell; *Margaritaria nobilis* (clavito) L.f., *Plectranthus amboinicus* (oreganon) (Lour.) Spreng. The oils got from *P. marginatum*, *H. suaveolens* and *P. racemosa*, showed no repellent effect, the rest showed significant repellency in varying degrees, with oils of *P. caruleocamum* and *M. nobilis* the most effective, showed a mean complete protection percentage against bites of *L. migonei* of 2 h. The *P. caeruleocanum* oil showed the long-acting protection time close to DEET, with a mean protection time over 4 h at concentrations of 50% and 2:30 h at 10%. Although *P. amboinicus* oil also presented repellent effect, it showed as well toxic effect irritant. The results showed an average for the first bite of *L. migonei* without any product of p1: 8.48 min and for second bite of p2: 13.69 min; while in the presence of *P. caruleocamum* oil at 100% p1: 183.68 min and p2: 284.50 min; a concentration of 50% p1: 129.01 min and p2: 250.81 min and 10% of p1: 31.65 minutes and p2: 164.99 min. The oils of *P. caruleocamum* and *M. nobilis* were effective repellent for more than 3 h against the bites of *L migonei.* The results suggest that the oils *P. caruleocamum* and *M. nobilis* could be potential candidates as natural repellents against *Leishmania* transmitters, however, it is necessary to carry on higher studies about their component and repellent activities.

241

ROLE OF MONOOXYGENASE, ESTERASE, GLUTATHIONE S-TRANSFERASE AND THE KNOCKDOWN RESISTANT (KDR) ALLELE IN CONFERRING PYRETHROID RESISTANCE IN *ANOPHELES GAMBIAE*

Lisa J. Reimer[1], Adama Sacko[2], Sekou Traore[2], Gregory Lanzaro[1], Anthony Cornel[1]

[1]University of California at Davis, Davis, CA, United States, [2]Malaria Research and Training Center, Bamako, Mali

The spread of insecticide resistance genes in *Anopheles gambiae* Giles sensu stricto threatens to compromise vector-based malaria control programs. A mutation in the voltage-gated sodium channel gene is known to confer knockdown resistance (kdr) to pyrethroids. In Mali, West Africa the kdr allele occurs at a high frequency in the S molecular form but is absent in the sympatric M form. In this study we examine the role of alternative resistance mechanisms in conferring resistance in both forms. This family level study analyzes the role of kdr and enzyme activity by P450 monooxygenase, esterase and glutathione s-transferase in conferring resistance in populations from villages with high and low insecticide use.

242

SPATIAL REPELLENT, CONTACT IRRITANT AND TOXICITY RESPONSES OF *AEDES AEGYPTI* TO PLANT DERIVED COMPOUNDS AS COMPARED TO DEET

Diana K. Riner[1], Paige B. Sachs[1], Nichole L. Achee[1], Kamal Chauhan[2], John P. Grieco[1]

[1]Uniformed Services University Of The Health Sciences, Bethesda, MD, United States, [2]USDA, Beltsville, MD, United States

Aedes aegypti, cosmopolitan in its distribution, is the primary vector of dengue and yellow fever. Control of the vector and vector-host interaction is critical for reducing disease transmission. Topical repellents are an important part of personal protective measures that are used to reduce human exposure to biting insects. The concerns with topical repellents, in particular the industry standard DEET, are their short residual life as well as toxicity in children from prolonged usage. As a result, new topical repellents need to be identified. Recently, a novel high-throughput screening system (HITTS) has been developed that allows for rapid screening of mosquito behavioral responses to chemical compounds. The assays test the chemical's toxicity, contact irritancy (movement away after contact with chemical) and spatial repellency (movement away without contact with chemical) characteristics. One advantage of this assay system is the ability to test mosquito behavior in the absence of a host source thus eliminating the need for human test subjects. This system has been used previously to screen for behavior modifying properties of DDT and alphacypermethrin and the data validated in field hut studies in Thailand. Using HITSS, we look at three concentrations of five plant-derived compounds including palmarosa oil, wild verbena, nepetalactam, davana oil, and karanj seed oil, which are known to have repellent properties and compared them with an internal standard, N,N-diethyl-3-methylbenzamide (DEET), to determine their impact on the behavioral responses of *Ae. aegypti*.

243

ANTI MOSQUITO CONTROL MEASURES TRENDS IN KINSHASA, DEMOCRATIC REPUBLIC OF THE CONGO DRC (2002-2007) BOBANGA LT, MULUMBA MP, UMESUMBU-BOBANGA ES

Iengu T. Bobanga, Madishala P. Mulumba

University of Kinshasa, Kinshasa, Democratic Republic of the Congo

Plasmodium falciparum against antimalarial drugs and Anopheles resistance against insecticides, made difficult any project of malaria control in perennial zone of transmission. Insecticide Treated Net (ITN) occupies an important place in the control strategy. However, for a poor population, the acceptability of this tool, its accessibility in financial aspect and its variable conditions of use from one environment to another, the effectiveness of selected insecticides explain the complexity of the malaria control. The objective of this study was to determine the place of bed nets, particularly insecticide treated nets (ITN), as control methods used spontaneously by households in Kinshasa against mosquitoes. KAP surveys (knowledge-attitude-practice) were carried out in Kinshasa in two periods, the first survey was carried in 2002 and than second in 2008 for each period more than 2.300 household were visited. The most significant interactions between the apprehended factors were identified by use of the log-linear analysis, and the respective odds ratio was calculated. Among the anti-mosquito methods used by households, in 2002 the insecticides came at the head with 50, 8% followed by the bed net 32,9%. In this economy, the ITN represented only 6, 8 %. Six years after insecticide was 69%, bed net 62, 3 % and ITN 51%. The differences between the two periods were significant. Among the four most significant inciting factors of the bed net use, at the head came the habitat (center, periphery) followed by comfort of the household (number of persons by household), the knowledge of the existence of ITN and the number of children under 5 years. The use of the bed net was proportional to the degree of the mosquito harmful effect, whose perception was higher in the center than in periphery of Kinshasa. The use of ITN is in progress in DRC but under the National Malaria control Program objective. And more effort must be done.

244

USING ELEMENTARY SCHOOL STUDENTS TO ESTABLISH A MOSQUITO LARVAE MONITORING SYSTEM

Sandy Hoar

George Washington University, Washington, DC, United States

This study was undertaken to develop an applied epidemiology pilot project to show students the relevance of public health to their lives, specifically monitoring for mosquito larvae as a herald to increasing malaria and dengue incidence and the effects of trash removal as remediation. Forty 5th and 6th grade students in rural eastern Mexico participated in a needs assessment and were given an interactive presentation on beginning epidemiology, including evaluating risk of disease in a population, monitoring and surveillance, and the life cycle of mosquitoes. The entire school of 100 students from 1st to 6th grade was divided into 6 teams. Each team was given a large plot of land adjacent to the school to map and then check for standing water. Any standing water was carefully checked for mosquito larvae. Each team was tasked with weekly monitoring of standing water and mosquito larvae. Two teams were tasked with trying to eliminate standing water by filling in divots and throwing trash.The needs assessment identified both trash and dengue as current regional problems. The students were able to draw and measure their plot of land. Standing water was identified and insects, though not mosquito larvae, were found in the water. The most standing water was found in discarded plastic. Students were enthusiastic about the project and stayed after school to complete the initial phase. The students identified trash and dengue as current problems and were able to identify why trash was important in their current beginning dengue infestation. The students identified serving their community as an important reason for conducting the surveillance. Future evaluation will include assessing change in attitude toward and a desire for a career in public health, evaluation of their weekly log books, and the presentation of results to local and regional officials. It will include expanding the number of plots monitored and establishing a monitoring system for cases of dengue or dengue and malaria.

245

SUSCEPTIBILITY OF *PLASMODIUM VIVAX* TO *ANOPHELES ANTHROPOPHAGUS* FROM DIFFERENT AREAS IN CHINA

Guoding Zhu[1], Qi Gao[1], Huayun Zhou[1], Julin Li[1], Feng Lu[1], Yaobao Liu[1], Sattabongkot Jetsumon[2]

[1]Jiangsu Institute of Parasitic Diseases, Wuxi, China, [2]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

This syudy was undertaken to compare the susceptibility of *Plasmodium vivax* to *Anopheles anthropophagus* (An.a) from Jiangsu,Guangdong, Liaoning province in China. Blood samples of patients in the vivax epidemic area in China were collected and fed to mosquitoes of An.a from different areas by using the artificial membrane feeding system *in vitro*. Mosquitoes were dissected on day 7-9 and day 14 after feeding. Oocysts and sporozoites were counted in stomach and glands of the mosquitoes. The mosquito of An.a from Jiangsu, Guangdong, Liaoning were simultaneouly fed with blood from 35 with *Plasmodium vivax* . I) The oocyst positive rate of An.a from Jiangsu,Guangdong, Liaoning on day 7- 9 were 68.57%, 60.00%, and 68.57%, as well as the sporozoite positive rate of them during the day 14 were 22.86%, 14.29%, and 22.86%, respectively. II) During the day 7 th after feeding, 228,235,228 mosquitoes of An.a from Jiangsu,Guangdong,Liaoning were dissected,the positive mosquito rate with the oocyst infection were 28.07%, 25.11%, 26.75%,respectively. During the day 14 th after feeding,150,142,135 mosquitoes of An.a from three areas were dissected, the positive mosquito rate with sporozoite infection were 10.67%,9.68%,13.67%,respectively. III) The mosquiotes' number dissected with infective grade ("+","++""+++""++++") of sporozoite of An.a from Jiangsu, Guangdong, Liaoning were 4, 3, 2, 7; 2, 2, 3, 7; 1, 6, 3, 8, respectively. In conclusion, An.a from Jiangsu, Guangdong, Liaoning was infective to parasites of *Plasmodium vivax* and there is no significant difference about the susceptibility of parasites to An.a from three areas.

246

IMPACT OF *ANOPHELES ARABIENSIS* FEEDING AND RESTING BEHAVIOUR IN ZOOPROPHYLAXIS FOR MALARIA CONTROL

Eliningaya J. Kweka, Aneth M. Mahande, Johnson M. Mahande

Tropical Pesticides Research Institute, Arusha, United Republic of Tanzania

The most important factor for effective zooprophylaxis in reducing malaria transmission is a predominant population of a strongly zoophilic mosquito, *Anopheles arabiensis*. The feeding preference behaviour of Anopheline mosquitoes was evaluated in odour-baited entry trap (OBET). Mosquitoes were captured daily using odour-baited entry traps, light traps and hand catch both indoor and in pit traps. Experimental huts were used for release and recapture experiment. The mosquitoes collected were compared in species abundances. *A. arabiensis* was found to account for over 99% of *An.* species collected in the study area in Lower Moshi, Northern Tanzania. In experimental release/capture trials conducted at the Mabogini verandah huts, *An. arabiensis* was found to have higher exophilic tendency (80.7%) compared to *An. gambiae* (59.7%) and *Culex spp*. (60.8%). OBET experiments conducted at Mabogini collected a total of 506 *An. arabiensis* in four different trials involving human, cattle, sheep, goat and pig. Odours from the cattle attracted 90.3% (243) compared to odours from human, which attracted 9.7% (26) with a significant difference at P =0.005. Odours from sheep, goat and pig attracted 9.7%, 7.2% and 7.3%, respectively. Estimation of HBI in *An. arabiensis* collected from houses in three lower Moshi villages indicated lower ratios for mosquitoes collected from houses with cattle compared to those without cattles.

HBI was also lower in mosquitoes collected outdoors (0.1-0.3) compared to indoor (0.4-0.9). In discussing the results, reference has been made to observation of exophilic, zoophilic and feeding tendencies of *An. arabiensis*, which are conducive for zooprophylaxis. It is recommended that in areas with a predominant *An. arabiensis* population, cattle should be placed close to dwelling houses in order to maximize the effects of zooprophylaxis. Protective effects of human from malaria can further be enhanced by keeping cattle in surroundings of residences.

247

A LARGE SEMI-FIELD SYSTEM FOR EXPERIMENTAL STUDY OF AFRICAN MALARIA VECTOR ECOLOGY AND CONTROL IN TANZANIA

Heather Ferguson[1], Kija R. Ng'habi[2], Sarah J. Moore[2], Issa Lyimo[2], Tanya L. Russell[2], Honorathy Urassa[2], Hassan Mshinda[2], Gerry Killeen[2], Bart G. Knols[2]

[1]University of Glasgow, Glasgow, United Kingdom, [2]Ifakara Health Research and Development Centre, Ifakara, United Republic of Tanzania

Medical entomologists increasingly recognize that the ability to make inferences between laboratory experiments of vector biology and epidemiological trends observed in the field is hindered by a conceptual and methodological gap occurring between these approaches which prevents hypothesis-driven empirical research from being conducted on relatively large and environmentally realistic scales. The development of Semi-Field Systems (SFS) has been proposed as the best mechanism for bridging this gap; which are defined as enclosed environments, ideally situated within the natural ecosystem of a target disease vector, in which all features necessary for its lifecycle completion are present. Although the value of SFS as a research tool for malaria vector biology is gaining recognition, only a few such facilities exist worldwide and all on relatively small scales (< 100 m^2). We describe the establishment of a 625 m^2 state-of-the-art SFS for large-scale experimentation on anopheline mosquito ecology and control within a rural area of southern Tanzania where malaria transmission intensity is amongst the highest ever recorded. The SFS was set up for a variety of research activities including mass-rearing for African malaria vectors under natural conditions, high throughput evaluation of novel mosquito control and trapping techniques, short-term assays of host-seeking behaviour and olfaction, and longer-term experimental investigation of anopheline population dynamics and gene flow within a contained environment that simulates a local village domestic setting. Preliminary observations indicate that realistic and repeatable observations of anopheline behaviour are obtainable within the SFS, and that habitat and climatic features representative of field conditions can be simulated within it. As work begins in the SFS in Ifakara and others around the world, we discuss the major opportunities and challenges to the successful application of this tool for malaria vector research and control.

248

SPATIO-TEMPORAL DYNAMICS OF IMMATURE CULICINES AND THEIR LARVAL HABITATS IN MWEA RICE SCHEME, KENYA

Ephantus J. Muturi

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

An ecological study was conducted to identify the diverse aquatic habitats in which culicine mosquitoes thrived in 3 study sites and explore the best strategies for mosquito control in Mwea Rice Scheme, Kenya. During the 11-month study period, 10 habitat categories and 11 culicine species mainly dominated by *Culex quinquefasciatus* (69.7%) and *Cx. annulioris* (19.7%) were identified. Two of the 11 culicine species; *Ficalbia plumosa* and *Uranotaenia* spp have not been reported previously in the study area. Rurumi had more habitat types than either of the other study sites but the least number of mosquito species. In contrast, Karima had the least number of habitat types but significantly higher counts of early instars than the other study sites. The counts of late instars and pupae did not vary significantly among study sites. The contribution of different habitat types to larval production varied markedly between seasons and among study sites. Paddies and canals were perennial contributors of culicine mosquito larvae while the other habitat types were important mainly during the wet season. Some habitat types such as ditches, seeps, marshes and fishpond were absent in some study sites but of great significance in other study sites. *Cx. quinquefasciatus* was positively associated with turbidity at all study sites and also negatively associated with emergent vegetation and distance to the nearest homestead in Karima, emergent vegetation in Kiuria and other aquatic invertebrates in Rurumi. *Cx. annulioris* was positively associated with emergent vegetation at all study sites and also with depth in Kiuria. These findings indicate that besides rice fields and associated habitats, a diversity of other aquatic habitats contribute to culicine adult mosquito production in the study area and that environmental factors that determine the occurrence of a particular mosquito species may vary significantly even among areas of similar land use. This information is critical when designing and implementing mosquito larval control programs.

249

IMPACT OF *PLASMODIUM FALCIPARUM* INFECTION ON THE EGGS HATCHING RATE AND THE LARVAL DEVELOPMENT TIME AMONG THE MOLECULAR FORMS M AND S OF *ANOPHELES GAMBIAE S.S*

Alpha S. Yaro, Amadou Guindo, Abdoulaye M. Touré, Adama Dao, Mamadou B. Coulibaly, Sekou F. Traoré

MRTC, Bamako, Mali

Infection of the major malaria vector of sub-saharan Africa, *Anopheles gambiae* by *Plasmodium falciparum* the most dangerous parasite species, may reduce eggs production. Reduction in egg production may occur when follicular cells are invaded by oocysts. In the current study, we aimed at measuring the impact of sporozoites presence on eggs hatching rate and larvae development time in M and S molecular forms of *An. gambiae* s.s. Females were allowed to single oviposit and then tested by ELISA for the presence of circumsporozoite protein. M and S molecular forms of *An. gambiae* s.s. were identified by PCR. Comparison analysis was performed between M and S molecular forms infected and uninfected by *P. faciparum*. There was no significant difference in eggs hatching rate M and S forms, regardless they are infected or not. Larvae development time was similar between M and S uninfected. In contrast, a two-day difference was found between the larvae development time in infected M and infected S forms (P=0.004). There was no difference in F1s sex ratios between M and S in both infected and uninfected comparison groups. The average size for F1s was statistically comparable between infected M and S. In conclusion we did not detect a major impact of the presence of sporozoites in M and S molecular forms of *An. gambiae* s.s. on their eggs hatching rate and the size of the adults F1s they produced. The difference of the development time between the F1s from M and S infected group should be double checked with a larger sample size.

250

MALARIA-INFECTED MOSQUITO MORTALITY IS AGE- AND *PLASMODIUM*-DENSITY DEPENDENT

Emma J. Dawes, Shijie Zhuang, María-Gloria Basáñez, Robert E. Sinden

Imperial College London, London, United Kingdom

Daily mortality is an important determinant of a vector's ability to transmit pathogens, influencing the probability of encountering infectious hosts, surviving the extrinsic incubation period, and transmitting the infection. Malaria transmission models typically assume that vector mortality is independent of age, infection status and parasite load. To test the validity of these assumptions and to ascertain the importance of parasite-induced vector mortality at various stages in the sporogonic cycle as a possible

cause of density-dependent regulation of parasite abundance, a series of three experiments were conducted. In each, four cages of *Anopheles stephensi* were fed on blood infected with different *Plasmodium berghei* ookinete densities (~500 fed female mosquitoes per cage), including a control, non-ookinete forming strain. Twice daily the number of dead mosquitoes in each group was recorded, and on alternate days a sample of live mosquitoes from each group were dissected to determine parasite density in both midgut and salivary glands. Survival analyses indicate that mosquito mortality is both age- and infection intensity-dependent. Mortality in lightly-infected mosquito groups did not differ significantly from that in the control groups, whereas increased, dose-dependent, mortality was recorded in the moderately- and heavily-infected mosquito groups. Implications for our understanding of the impact of parasite density on malaria transmission dynamics will be discussed.

251

COMPARATIVE CAPTURE RATE RESPONSES OF MOSQUITO VECTORS TO LIGHT TRAP AND HUMAN LANDING COLLECTION METHODS

Donald Barnard, Gregory Knue

USDA-ARS, Gainesville, FL, United States

Landing rates (LR) of female *Anopheles quadrimaculatus, Culex nigripalpus, Cx. quinquefasciatus*, *Ochlerotatus triseriatus* and *Aedes albopictus* on human hosts were compared with capture rates responses by the same species to CDC-type light traps (LT) augmented with CO_2. A significant relationship between seasonal responses to LR and LT collection methods was observed for *An quadrimaculatus*. Diel responses to LT and LR were related for *Culex* spp. only. The modes of daily activity indicated by each collection method compared poorly within species with such differences greatest for *Ae. albopictus* and *Oc. triseriatus*. Capture efficiency indices showed that temporal responses to LT and LR are not congruent and that the former typically underestimates LR. A statistical model is used to identify specific times within the diel period that LT can be used to sample the adult mosquito population and the results translated precisely in terms of the numeric mosquito landing rate on humans.

252

EVALUATION OF THE INDOOR AND OUTDOOR BLOOD FEEDING BEHAVIOR OF *ANOPHELES ARABIENSIS* IN AN AREA WITH HIGH INSECTICIDE TREATED BED NET USE IN SOUTHERN ZAMBIA

Christen M. Fornadel[1], Shadrack Habbanti[2], Mulenga Musapa[2], Julie A. Clennon[1], Douglas E. Norris[1]

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

Anopheles arabiensis, the primary vector of *Plasmodium falciparum* in Macha, Zambia, is known to display varying degrees of exophilic and exophagic behavior. The extent of these behaviors was evaluated with paired indoor/outdoor human landing catches (HLCs) and outdoor cattle-baited collections. Results concur with previous studies that *An. arabiensis* in Macha is highly anthropophilic. Additionally, the effect of insecticide treated bed nets (ITNs) on entering behavior, as well as the efficacy of human-baited collections, was examined by comparing the performance of HLCs with CDC light traps hung in sleeping houses with and without ITNs. While no apparent repellant effect was observed, more *An. gambiae* complex mosquitoes were caught in HLCs conducted outdoors. It will be important to continue to monitor *An. arabiensis* blood feeding behavior in the region as exophagic behavior has the potential to circumvent the protective effects of ITNs.

253

THE MITOCHONDRIA CYTOCHROME OXIDASE 1 DNA SEQUENCES DEFINE ECOLOGICAL DISTRIBUTION OF *ANOPHELES GAMBIAE* SPECIES COMPLEX IN GHANA

Dziedzom K. de Souza[1], Michael D. Wilson[2], Charles A. Brown[2], Bernard W. Lawson[3], Daniel A. Boakye[2]

[1]Noguchi Memorial Institute for Medical research/Department of Theoretical and Applied Sciences, Kwame Nkrumah University of Science and Technology, Accra/Kumasi, Ghana, [2]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [3]Department of Theoretical and Applied Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

The mitochondria cytochrome oxidase 1 (CO1) gene sequences of insects have been used in studies to delineate species and members of sibling species complexes, but not for *Anopheles gambiae* complex the vectors of malaria and lymphatic filariasis (LF) in West Africa. In the sub region malaria occurs everywhere and transmission is perennial with marked peaks during the wet season. However studies have shown that the same vector species differ in their vectorial importance from place to place. Lymphatic filariasis has a focal distribution despite the fact that it is also transmitted by the same *Anopheles* vectors. Moreover where the two diseases occur together a negative spatial association between them has been observed. In our attempts to investigate the above phenomena, we decided to study the diseases vectors' CO1 sequence variation at 14 sites in 5 bio-ecological zones in Ghana. About 650bp of the CO1 DNA sequences of 31 *An. gambiae* s.s and 3 *An. melas* were amplified by PCR and sequenced, and these formed the database for the phylogenetic analysis. The phylogenetic relationships were inferred using the Neighbourhood-Joining and Kimura 2-parameter methods using MEGA4 software. These preliminary results obtained clearly reveal clustering along ecological lines. That the coastal savanna mosquito sequences clustered together with those of the Sudan savanna in the northern half of Ghana, makes us believe that this is a result of climate changes which bisected the Guinea-Congo Forest belt, and also created the Dahomey Gap during the Pleistocene period.

254

IMPACT OF EXPOSURE TO *ANOPHELES* BITES ON THE DEVELOPMENT OF HUMAN ANTIBODY RESPONSE TO *PLASMODIUM FALCIPARUM* IN CHILDREN LIVING IN MALARIA ENDEMIC AREA

Jean Biram Sarr

IRD, Dakar, Senegal

Numerous ecological and epidemiological factors could modulate the anti-malaria immunity. Among these factors, the exposure to *Anopheles* bites and, especially the active components of *Anopheles* saliva, could play a key role on the development of immune response to *Plasmodium falciparum* in exposed individuals. The objective of the study was to evaluate the impact of the exposure to Anopheles species on the development of antibody (Ab) response against *P. falciparum* in children living in malaria area. A multi-disciplinary study was conducted in two Senegalese villages where malaria transmission/exposure was ensured by dissimilar *Anopheles* species (Mboula: low exposure to *An. gambiae* s.l; Gankette: high exposure to An. funestus). In each village, epidemiological, parasitological, entomological and immunological data was followed in children (1-9 years; n=120). IgG, IgG1, IgG3 response directed to *P. falciparum* whole schizont extract (WSE) and specific antigens (all stages) were evaluated before (June), at the peak (September) and after (December) the period of transmission. In Mboula, the peak was followed by a considerable increase of anti-WSE IgG levels whereas low and constant specific IgG response was observed through the year in Gankette. Interestingly, anti-WSE IgG1 levels were significantly higher in Mboula whereas specific IgG3 response predominated in Gankette. These results suggest that specific IgG and isotype IgG responses could be

regulated according to the nature (Anopheles species) and/or the intensity of exposure to Anopheles bites. In addition, IgG response to several antigens (CSP, TRAP, GLURP, LSA1, LSA3) progressively decreased from June to December in children negative for malaria infection. It suggested an immuno-suppression of IgG responses to specific antigens during the season of exposure to Anopheles bites. Altogether, this study shows that the development of anti-malaria Ab response was profoundly different according to areas where the exposure is dependent to the intensity and/or species of *Anopheles*. The influence of *Anopheles* saliva could be thus involved in the observed immune regulation. This study represents one first immuno-epidemiological approach on the influence of exposure to mosquito bites on the development of anti-pathogen immunity.

255

HOST DENSITY EFFECTS ON FEEDING BEHAVIOR OF *CULEX QUINQUEFASCIATUS* SAY (DIPTERA: CULICIDAE)

Ivo M. Foppa[1], Kevin A. Caillouet[1], Sarah R. Michaels[1], Ricardo Cortez[2], Dawn M. Wesson[1]

[1]Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States, [2]Tulane University School of Science and Engineering, New Orleans, LA, United States

Epidemiological theory suggests that an increase in feeding density of vector mosquitoes on amplification hosts intensifies mosquito-borne transmission. Feeding density is a function of host density (and density of vector mosquitoes). Thus, a decline in local host numbers, as sometimes associated with West Nile virus (WNV) epizootics, could increase transmission intensity if numbers of vector mosquitoes are held constant. It is, however, largely unknown how WNV vectors such as *Culex quinquefasciatus* Say, react to abrupt changes in host density. The nature of that response may have important implications for transmission dynamics and dispersion of WNV. We were particularly interested in the question whether such mosquitoes actively disperse when hosts become locally sparse. We therefore designed a series of indoor experiments simulating low local host density (e.g. after a catastrophic localized WNV epizootic), with alternative hosts available at some distance. Briefly, we will simultaneously expose laboratory reared *Cx. quinquefasciatus* to different densities of chickens kept approximately 25m apart. Mosquitoes will be released intermediately between the two cages as well as from the location with lower host density. Number of chickens in the two cages will be varied from 1 to 20. Engorged mosquitoes will be retrieved from the cages and counted. The number of engorged mosquitoes will statistically modeled as a function of mosquitoes released, numbers of chickens in the two cages, and distance between release and the two cages. The results from these experiments will offer new insight into the local transmission dynamics and dispersal of WNV as a function of changing host densities.

256

CULEX VECTOR COMPETENCE DOES NOT ENABLE OUTBREAKS OF WEST NILE VIRUS IN CALIFORNIA

W. Reisen, C. Barker, Y. Fang

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

Since the invasion of California by West Nile virus (WNV) in 2003, the vector competence for the NY99 strain has been monitored for *Culex tarsalis* Coquillett, *Cx. pipiens quinquefasciatus* Say, *Cx. p. pipiens* L., and *Cx. stigmatosoma* Dyar populations from four areas: deserts of Coachella Valley, densely urbanized maritime Los Angeles, southern San Joaquin Valley in Kern County and southern Sacramento Valley near Davis in Yolo and Sacramento Counties. *Cx. stigmatosoma* was the most competent vector species, following by *Cx. tarsalis* and the *Cx. pipiens* complex. The median infectious dose (ID_{50}), a measure of mesenteronal susceptibility, ranged between 5 and 8 log10 plaque forming units per mL and was not correlated with human case incidence or summer *Culex* species infection rates. Odds ratios comparing non-outbreak years to referent outbreak years were variable and failed to show a distinct pattern for *Cx. tarsalis* or *Cx. pipiens* complex females. Apparently factors other than midgut susceptibility within the ranges we measured enabled WNV outbreaks in California. In addition, *Culex* populations remained competent for St. Louis encephalitis virus, indicating that the disappearance of this virus was not related to a loss of susceptibility.

257

POTENTIAL FOR NORTH AMERICAN MOSQUITOES TO TRANSMIT RIFT VALLEY FEVER VIRUS

Michael J. Turell[1], William C. Wilson[2], Kristine Bennett[2]

[1]U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, United States, [2]Arthropod-Borne Animal Diseases Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Laramie, WY, United States

The recent outbreaks of disease caused by Rift Valley fever virus (RVFV) in Kenya, Mauritania, Yemen, Tanzania, Somalia**,** and Madagascar indicate the potential for RVFV to cause severe disease in both humans and domestic animals and its potential to be introduced into new areas, possibly even North America. Because mosquito control methods are often species specific, measures that are effective for one species may have little or no effect on others. Thus, it is important to determine which mosquito species are capable of transmitting RVFV so that appropriate control measures can be instituted rapidly and efficiently, should this virus be introduced into North America. Therefore, we evaluated *Aedes dorsalis, Ae. vexans,* and *Culicoides sonorensis* from the midwestern United States for their ability to serve as potential vectors for RVFV. Specimens were allowed to feed on adult hamsters inoculated with RVFV, incubated for 7-21 days at 26°C, and then allowed to refeed on susceptible hamsters and tested to determine infection, dissemination, and transmission rates. None of the species tested was able to transmit RVFV efficiently under laboratory conditions despite feeding on a hamster with a viremia of $10^{8.8}$ plaque-forming units/ml of blood. Although *Ae. dorsalis* was the most susceptible to infection (78%) and had the highest dissemination rate (33%), this species had a salivary gland barrier and rarely transmitted RVFV by bite. In contrast, only 26% and 6% of *Ae. vexans* became infected and developed a disseminated infection, respectively. However about 50% of those with a disseminated infection transmitted virus by bite. None of the *C. sonorensis* became infected, even after intrathoracic inoculation, indicating that this species would not be a competent vector. In addition to laboratory vector competence, factors such as seasonal density, feeding preference, longevity, and foraging behavior also need to be considered when determining the role these species could play in RVFV transmission.

258

MOSQUITO, *AEDES AEGYPTI,* INFESTATION CAUSES CD4+ T-CELLS RESPONDING TO COGNATE ANTIGEN TO DEVELOP THE CAPACITY TO EXPRESS INTERLUKIN-4

V. D. Boppana[1], F. J. Alarcon-Chaidez[1], S. Thangamani[1], A. J. Adler[2], S. K. Wikel[1]

[1]Department of Immunology, University of Connecticut Health Center, Farmington, CT, United States; Current Address: Department of Pathology, University of Texas Medical Branch, Galveston, TX, United States, [2]Department of Immunology, University of Connecticut Health Center, Farmington, CT, United States

Mosquito saliva contains biologically active molecules essential for blood feeding and pathogen transmission. *Aedes aegypti* saliva contains inhibitors of host hemostasis, modulators of host immune defenses and numerous molecules of unknown function. Stimulation and modulation of host immunity are logical consequences of exposures to mosquito bites. A clonotypic population of T-cell receptor transgenic CD4+ cells with an extreme Th1 bias was adoptively transferred into BALB/c mice to determine if *Ae. aegypti* bites induce expression of the Th2 effector cytokine IL-4. Recipient mice were exposed to 5 or 10 bites on days -1 and +1 with adoptive transfer of CSFE-labeled clonotypic CD4+ T-cells on

day 0 followed by intradermal injection of 200 µg of cognate antigen, influenza hemagglutinin (HA), one hour later. A second group of mice received primary and secondary infestations, as described, which were separated by 14 days. Lymph nodes draining bite sites were harvested at day +4 and cells restimulated with 100 µg HA for 5 hours. Clonotypic T-cells were immunophenotyped; their CFSE dilution assessed; and, stained for intracellular cytokines. Mosquito bites induced clonotypic HA-specific CD4+ T-cells to undergo significant proliferative responses as visualized by the dilution of CFSE-fluorescence. Infestation with mosquitoes caused a small but significant increase in percentage of clonotypic T-cells expressing IL-4. A second exposure to mosquito bites resulted in a significant decrease in percentage of clonotypic CD4+ T-cells induced to express IL-4 compared to cells obtained following a primary exposure to mosquito bites. Intradermal inoculation of *Ae. aegypti* salivary gland extract programmed IL-4 expression in a similar manner to that of mosquito bites.

259

A REVIEW OF *ANOPHELES GAMBIAE* LARVAL ECOLOGY AND ITS IMPLICATIONS FOR CONTROL

Ulrike Fillinger[1], Silas Majambere[1], Bryson Ndenga[2], Khadija Kannady[3], Steven W. Lindsay[1]

[1]Durham University, Durham, United Kingdom, [2]Kenya Medical Research Institute, Kisumu, Kenya, [3]Urban Malaria Control Program, City Council, Dar es Salaam, United Republic of Tanzania

We are now at a critical time in the history of malaria control in Africa. For the first time in a generation reports of malaria declining have become common. The present strategy is sensibly targeted at immediate improvements in control using insecticide-treated nets, indoor-residual spraying and the prompt and effective treatment of clinical malaria. In order to maintain the hard won gains and to aim for even further reductions of malaria transmission, more tools have to be applied in an integrated fashion. Consequently, there has been a growing interest in attacking the aquatic stages of malaria vectors using larvicides in conjunction with environmental management. Successful anti-larval interventions targeting the most notorious malaria vector require a sound understanding of its larval ecology. Here we review a number of frequently cited dogmas of *Anopheles gambiae* larval ecology based on findings from recent large-scale longitudinal studies in The Gambia, Kenya and Tanzania and discuss their implications for the effectiveness of larval control intervention.

260

EVALUATION OF FEMALE *AEDES AEGYPTI* RESTING PREFERENCES USING A NOVEL LABORATORY ASSAY

Nicole L. Achee[1], Suppaluck Polsomboon[2], John P. Grieco[1]

[1]Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [2]Katsetsart University, Bangkok, Thailand

Spread by *Aedes aegypti*, a mosquito that prefers to feed on humans, dengue fever and dengue hemorrhagic fever are a major public health problem. As part of a larger research program focused on dengue vector control, an assay has been designed to quantify resting behavior patterns of *Ae. aegypti* under laboratory conditions. Identifying key variables that influence mosquito resting preferences is vital in developing new strategies for adult *Ae. aegypti* prevention inside homes. The objective of the current study was to quantify the preference of two geographically unique *Ae. aegypti* strains to rest on dark vs. light material. In addition, changes in resting patterns were evaluated in response to: 1) varying coverage of dark material; 2) horizontal vs. vertical contrast; 3) material texture; and 4) chemical irritant. Results from these experiments will be used to guide experimental hut validation studies in both Peru and Thailand.

261

HOST PREFERENCE OF POTENTIAL WEST NILE VIRUS VECTORS IN GUATEMALA

Rebekah J. Kent[1], Maria Eugenia Morales[2], Nicholas Komar[1]

[1]Centers for Disease Control and Prevention, Fort Collins, CO, United States, [2]Universidad del Valle de Guatemala, Guatemala City, Guatemala

Serologic evidence of West Nile virus (WNV) was discovered in Guatemala in 2003, and WNV has since been isolated from at least three species of *Culex (Culex)* mosquitoes. The clay-colored robin (*Turdus grayi*), great-tailed grackle (*Quiscalus mexicanus*), and domestic chicken (*Gallus gallus*) have been identified as frequently infected resident avian hosts within the Department of Izabal. However, the relative importance of these and other tropical species to enzootic transmission of WNV is unknown. Therefore, we characterized the blood feeding behavior of multiple *Culex* species in Guatemala and related mosquito host preferences to avian host competence, seroprevalence, and relative abundance. Engorged mosquitoes were aspirated from rural and urban habitats during the wet (July) and dry (December) seasons in the Department of Izabal in 2007, and the relative abundance of humans, domestic animals and wild birds were estimated for each site. Blood meals were identified by DNA sequencing of mitochondrial cytochrome oxidase 1 and cytochrome b. The relative contribution of various bird species to WNV transmission was modeled for different habitats and mosquito species, and mosquito host preference was examined by comparison of forage ratios. Our data demonstrate the complexity of WNV ecology in Guatemala, with numerous *Culex* species potentially involved in virus transmission, and differences in mosquito species composition and blood feeding patterns across habitats and seasons. The spatial and seasonal blood feeding behavior for multiple mosquito species will be discussed.

262

INCREASING FLUOROQUINOLONE RESISTANCE IN *SALMONELLA TYPHI* IN ONTARIO, 2002-2007

Shaun K. Morris[1], Susan E. Richardson[1], Laura Sauve[2], E. Lee Ford-Jones[1], Frances Jamieson[3]

[1]The Hospital For Sick Children, Toronto, ON, Canada, [2]British Columbia Children's Hospital, Vancouver, BC, Canada, [3]Central Public Health Laboratory, Toronto, ON, Canada

Typhoid fever, caused by *Salmonella typhi*, remains an important global public health issue. Though the risk of acquiring the disease in developed countries is low, imported cases are not uncommon, reflecting travel from endemic areas, especially the Indian subcontinent. Until the early 1990s, the treatment of choice for typhoid fever was ampicillin, choloramphenicol, or trimethoprim-sulfamethoxazole. However, because of increasing resistance to these drugs, ciprofloxacin replaced them as the drug of choice in the early 1990s. Reduced susceptibility and in-vitro resistance to ciprofloxacin followed shortly thereafter, accompanied by clinical treatment failures. This study was undertaken to document changes in antibiotic sensitivies of *S. typhi* in Ontario between 2002 and 2007. We reviewed the antibiotic susceptibility patterns of all isolates of *S. typhi* in Ontario, Canada from January 2002 to December 2007. In Ontario, all isolates of S. typhi are sent to the Central Public Health Laboratory (CPHL) for confirmatory identification and antibiotic susceptibility testing. We identified a total of 381 unique cases over the five year period (50-73 cases per year). Of the 381 cases, 171 were female, 164 were male, and no gender was identified for 33 cases. Age of the patients ranged from less than 1 to 102 years of age (median age of 20 years). While resistance patterns for ampicillin, trimethoprim-sulfamethoxazole, third generation cephalosporins (cefotaxime until May 2005 and ceftriaxone from June 2005 to present), and chloramphenicol remained stable, nalidixic acid resistance rose sharply between 2003 and 2005, and has remained at approximately 80% of isolates since 2005. The significant and sustained increase in nalidixic acid-resistant *S. typhi* suggests that ciprofloxacin should no longer be used as the drug

of choice for the empiric treatment of typhoid fever in Ontario. Similar recommendations hold true for other settings including the United States, in which the majority of imported cases originate in travelers and immigrants from the Indian subcontinent, or where the prevalence of nalidixic acid resistance in isolates of *S. typhi* is known to be high. Therapeutic alternatives include a third generation cephalosporin, or azithromycin for those with a β lactam allergy.

263

VARIABILITY IN MEASUREMENT OF HAND CONTAMINATION: IMPLICATIONS FOR EVALUATING HAND HYGIENE PROMOTION PROGRAMS

Pavani Kalluri Ram[1], Iqbal Jahid[2], Amal K. Halder[2], Benjamin Nygren[3], M Sirajul Islam[2], Stewart P. Granger[4], John W. Molyneaux[5], Stephen P. Luby[2]

[1]University at Buffalo, Buffalo, NY, United States, [2]ICDDR,B: International Centre for Diarrhoeal Diseases Research, Dhaka, Bangladesh, [3]Emory University, Atlanta, GA, United States, [4]Unilever RandD Port Sunlight, Bebington, United Kingdom, [5]Water and Sanitation Program, The World Bank Group, Washington, DC, United States

Hand hygiene programs frequently promote washing hands at critical times, such as before feeding a child. Measuring hand contamination at critical times can be logistically challenging and requires costly structured observation. To determine the utility of random microbiological testing of hands for assessing handwashing behavior, we examined whether hand contamination measured at random predicts contamination measured at critical times. Participating mothers of children < 2 years old in rural communities of Bangladesh rinsed both hands in a sterile bag containing 200 ml of ringer's solution. Standard membrane filtration was used to quantify colony forming units (CFU) of fecal coliforms and *Escherichia coli*. We calculated geometric means and absolute differences between results of random and critical time sampling for each mother. Hand rinses were obtained from 49 mothers at one random and at one critical time. Critical times included food preparation (57%) and feeding a child (18%). The geometric mean of fecal coliforms was 371 CFU/100 ml at random, and 2785 CFU/100 ml during the critical time. The geometric mean of *E. coli* was 24 CFU/100 ml at random, and 53 CFU/100 ml during the critical time. There was no correlation between random and critical time samples with respect to counts of fecal coliforms (R=.16, p=.26) or *E. coli* (R= -0.13, p=.38). The mean absolute difference in log-transformed results between random and critical time sampling was 1.3 (SD 1.4) for fecal coliforms and 1.1 (SD 1.1) for *E. coli*. We found no significant correlations and large absolute differences between levels of hand contamination at random and at critical times, demonstrating variability in contamination of the same subject's hands within the span of hours. Variability may be based on duration since last handwashing or last fecal contact. This degree of variability suggests that single random hand rinses are not valid proxy measures for handwashing behavior.

264

A PHASE II, OBSERVER BLIND, RANDOMIZED, CONTROLLED STUDY TO EVALUATE THE SAFETY, IMMUNOGENICITY AND IMMUNOLOGICAL MEMORY OF A BOOSTER DOSE OF A NEW GROUP A MENINGOCOCCAL CONJUGATE VACCINE (MENAFRIVAC ™) IN HEALTHY AFRICAN CHILDREN PREVIOUSLY PRIMED AT 12-23 MONTHS OF AGE

Olubukola T. Idoko[1], Brown J. Okoko[1], Samba Sow[2], Milagritos Tapia[2], Marie-Pierre Preziosi[3], Elisa Marchetti[4], Fadima C. Haidara[2], Lionel Martellet[5], Julie Chaumont[5], Varsha Parulekar[6], Brian Plikaytis[7], Ray Borrow[8], George Carlone[9], Richard Adegbola[1], Jean-Marie Preaud[5], Prasad Kulkarni[10], Subhash Kapre[10], Suresh Jadhav[10], Musa Hassan-King[5], Marc LaForce[5], Simonetta Viviani[5], Helen Findlow[8], Cheryl Elie[9]

[1]Medical Research Council, Banjul, Gambia, [2]Centre pour les Vaccins en Développement CVD-Mali, Bamako, Mali, [3]Meningitis Vaccine Project, Initiative for Vaccine Research, WHO, Geneva, Switzerland, [4]Meningitis Vaccine Project, PATH, Ferney-Voltaire, France, [5]Meningitis Vaccine Project, PATH, Ferney-Voltaire, France, [6]iGATE Clinical Research Int., Mumbai, India, [7]Centers for Disease Control and Prevention, Atlanta, GA, United States, [8]Vaccine Evaluation Unit, Health Protection Agency, Manchester, United Kingdom, [9]Centers for Disease Control and Prevention, Atlanta, GA, United States, [10]Serum Institute of India Ltd (SIIL), Pune, India

Over the last century, group A meningococci have remained unique in its ability to cause epidemics in the African meningitis belt. The Meningitis Vaccine Project was funded in 2001 to eliminate meningococcal epidemics in Sub-Saharan Africa through accelerated development and introduction of meningococcal group A (MenA) conjugate vaccine. As reported previously, a new MenA conjugate vaccine, was found safe and immunogenic with durable immunity when tested in a Phase I study in Indian adults. In a phase II study performed in African toddlers aged 12 -23 months, the MenA conjugate vaccine elicited 20-fold higher serum bactericidal antibody assay (rSBA) titres than a polysaccharide vaccine. We report here the results of the booster study. A total of 601 subjects aged 22-33 months who had received 10 month earlier either the MenA conjugate vaccine (201), meningococcal polysaccharide vaccine or Haemophilus *influenzae* type b vaccine, were randomised by each vaccination group to receive a single intramuscular injection of either MenA conjugate vaccine (PsA-TT), 1/5 dose of polysaccharide vaccine (PsACWY) or *Haemophilus influenzae* type b vaccine (Hib). Blood samples were obtained just prior to immunization, 7 days and 4 weeks later for rSBA and anti-polysaccharide group A IgG levels. PsA-TT vaccine showed a safety and reactogenicity profile similar to Hib and PsACWY vaccines when administered as a booster. Persisting antibody levels were significantly higher in the PsA-TT group, than in the other groups**.** Response to the boost immunization with the reduced dosage of PsACWY was significantly higher at both 7 and 28 days in the group who had initially received PsA-TT than in the group who had initially received PsACWY when all predefined rSBA and ELISA endpoints were considered. Response to PsA-TT booster at 28 days was very impressive in the PsA-TT/PsA-TT vaccine group as well as in the PsACWY/PsA-TT vaccine group. The vaccine was also highly immunogenic in naive children. In conclusion, this study showed that one dose of the new Men A conjugate vaccine administered in the second year of life effectively primes for immunological memory. Antibody persistence and boostable responses elicited by the new vaccine are in accordance with the characteristic features of a conjugate vaccine. It is expected that the widespread use of this vaccine in 1-29 year olds will eliminate group A meningitis epidemics form Sub-Saharan Africa.

265

DISCOVERY OF NEW *BARTONELLA* SPECIES IN BATS FROM KENYA

Michael Y. Kosoy[1], Ying Bai[1], Ivan V. Kuzmin[2], Michael Niezgoda[2], Richard Franka[2], Bernard Agwanda[3], Robert F. Breiman[4], Charles E. Rupprecht[2]

[1]Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, CO, United States, [2]Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Mammalogy Section, National Museum of Kenya, Nairobi, Kenya, [4]Global Diseases Detection Division, CDC in Kenya, Nairobi, Kenya

We report results of the first study to investigate the presence and diversity of *Bartonella* species in bats (*Chiroptera*) collected from different locations across Kenya. Whole blood from 120 bats of nine species was cultured for bartonella. Bartonellae were isolated from 24 bats (20%) including 15 of 38 (39.5%) *Eidolon helvum,* 4 of 62 (6.5%) *Rousettus aegyptiacus,* and 5 of 20 (25%) *Miniopterus* species. Sequencing of the citrate synthase gene of the isolates demonstrated a diverse assemblage of bartonella strains. Phylogenetically isolates clustered in conjunction with the host bat species. All isolates from *R. aegyptiacus* clustered in a monophyletic group that may represent a new *Bartonella* species. *E. helvum* harbored up to four *Bartonella* species that have not been documented previously. All but one isolate obtained from *Miniopterus* species (*M. natalensis* and *M. africanus*), were clustered together, and were distinct from all known *Bartonella* species. In addition, one isolate from *M. natalensis* was relatively similar to bartonella found in the cardiac tissue of bat *Myotis mystacinus* from England. The finding of *Bartonella* species in a high proportion of apparently healthy bats roosting in close proximity to humans in Kenya suggests the need to investigate whether these agents might be responsible for human illnesses in Kenya and elsewhere in Africa. Coupled with similar findings in other mammals, especially rodents, surveillance is justified for human bartonellosis, as a potentially important emerging zoonosis, among patients with unexplained febrile illness.

266

ANTIMICROBIAL SUSCEPTIBILITIES OF CLINICAL HUMAN ISOLATES OF *LEPTOSPIRA* FROM EGYPT

Clinton K. Murray[1], Guillermo Pimentel[2], Tina Parker[3], Miriam L. Beckius[1], Ahmed Samir[4], Bassem Abdel Rhman[2], Katrin Mende[5], Renee L. Galloway[6], Duane R. Hospenthal[1]

[1]Brooke Army Medical Center, Fort Sam Houston, TX, United States, [2]US NAMRU-[3], Cairo, Egypt, [3]National Institute of Health, Bethesda, MD, United States, [4]Cairo University, Cairo, Egypt, [5]Infectious Disease Clinical Research Program, Fort Sam Houston, TX, United States, [6]Centers for Disease Control and Prevention, Atlanta, GA, United States

Leptospirosis is a known cause of acute febrile illness in Egypt; however, there is limited information documenting the identity of endemic serovars or the antimicrobial susceptibility of these serovars. This study characterizes the *Leptospira* recovered from bloodstream cultures of febrile patients, including the determination of infecting serovar and antimicrobial resistance (AR) profiles. Patients presenting to 4 different hospitals located in Egypt (Cairo, Alexandria, Aswan and Mahalla) from 2005-2007 were enrolled if they had fever, age ≥ 4 years, and no identified cause of fever such as diarrhea, pneumonia, or a clinical diagnosis of typhoid fever or brucellosis. *Leptospira* were cultured from blood using EMJH media. Antimicrobial activity of penicillin G, ampicillin, cefotaxime, ceftriaxone, cefepime, imipenem-cilastatin, azithromycin, clarithromycin, ciprofloxacin, levofloxacin, moxifloxacin, tetracycline and doxycycline was determined by broth microdilution testing. Strain relatedness and serovar determination was performed by pulsed-field gel electrophoresis (PFGE) using NotI to digest bacterial DNA. A total of 2441 patients were enrolled with 98 (4.0%) having acute leptospirosis as determined by culture, molecular testing, or 4-fold rise in or single MAT titer of 1:800 or greater. There were 45 cultures available for PFGE and AR testing from Alexandria (8), Aswan (10), and Mahalla (27). PFGE testing revealed 2 species: *Leptospira interrogans* (44) and *L. kirschneri* (1). There were 7 serovars present: Icterohaemorrhagiae (13), Bataviae (10), Pomona (9), Pyrogenes (6), Grippotyphosa (4), Wolfii (2), Canicola (1**).** On AR testing, there was no difference in resistance profiles between serovars or regions of Egypt in which the isolates were recovered. The most active antimicrobial agents are β-lactams, macrolides, and fluoroquinolones. In conclusion, one primary species, but varying serovars are responsible for leptospirosis in the Nile region of Egypt. No *Leptospira* were recovered from Cairo. The antimicrobial agents with the greatest *in vitro* activity are consistent with other studies of *Leptospira*.

267

GENETIC DIVERSITY OF *ORIENTIA TSUTSUGAMUSHI* FROM FEBRILE PATIENT IN UPPER REGIONS OF THAILAND DURING 2004-2007

Pimmada Jeamwattanalert, Toon Ruang-areerate, Piyanate Sunyakumthorn, Carl J. Mason, Jariyanart Gaywee

Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

Scrub typhus caused by gram negative obligate intracellular bacterium, *Orientia tsutsugamushi*, is a widely endemic disease in Asian Pacific regions, including Thailand. The disease is clinically difficult to diagnose and can be lethal if left without appropriated treatment. To develop a sensitive and specific diagnostic tool as well as an effective vaccine for *O. tsutsugamushi* strains circulating in Thailand, it is necessary to understand their genetic diversity and phylogenetic relationship. Utilizing PCR, we have screened 607 febrile patients' blood collected from upper regions of Thailand during 2004-2007. Fragment of *O. tsutsugamushi* specific gene encoding major protein antigen, 56 kDa was detected in 51 cases (8.4%). Amplified fragments spanning over 3 major variable regions of 56 kDa gene have been sequenced and analyzed. Resulting 56 kDa phylogenetic tree demonstrated that detected *O. tsutsugamushi* clustered into 10 clusters. Four clustered with previous reported groups, Gilliam, Kato, TA 716 and LA1, whereas six could probably form new diverse groups. No Karp-like strain was found. The group of *O. tsutsugamushi* will make a choice of reputative strains of each assemblage that would be used as a model of Thai strains for focusing on development of specific and sensitive diagnosed tool and following by vaccine development next.

268

SPATIAL CLUSTERING BY DISEASE SEVERITY AMONG ROCKY MOUNTAIN SPOTTED FEVER CASES IN THE UNITED STATES, 2001 - 2005

Jennifer Adjemian, John Krebs, Eric Mandel, Jennifer McQuiston

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

Rocky Mountain spotted fever (RMSF), caused by *Rickettsia rickettsii*, is the most common fatal tick-borne disease in the United States. The clinical presentation of RMSF ranges from mild to severe infection with life-threatening complications. National surveillance suggests that the mean incidence rate for RMSF is increasing, though with apparent variation in disease severity by geographic location. To evaluate whether severe RMSF infections cluster geographically, we performed a spatial analysis of U.S. cases. RMSF cases reported to the Centers for Disease and Prevention (CDC) by Case Report Forms from 2001-2005 were included in the analysis. Cases were geo-coded by county and classified by three levels of disease severity: 1) cases reporting hospitalizations, complications and/or fatalities; 2) cases reporting complications and/or fatalities; and 3) only cases reporting fatalities. SaTScan™ was then used to detect spatial clusters with significantly greater or smaller rates of severe outcomes relative to the respective mean national rates (p value ≤ 0.05). Overall, 4,533 RMSF cases were reported from 2001-2005, of which 1,089 were hospitalized (168 with reported complications), and 23 were fatal. Significant clusters of 6 deaths (p=0.05, RR=11.4)

and of 19 hospitalizations with complications (p=0.02, RR=3.45) were detected in southwest Tennessee. Two clusters of mild RMSF cases were identified in southern and north-central North Carolina relative to national hospitalization (p=0.001, RR=0.62) and hospitalization with complication (p=0.001, RR=0.45) rates, respectively. Of all RMSF-associated hospitalizations, 20% were in eastern Oklahoma (p=0.023, RR=1.43). Significant clusters of both increased and decreased rates of severe outcomes in RMSF cases reported here are likely the product of complex interactions among biological, ecological, and anthropogenic factors that vary by geographic location. These observed variations could be due to the presence of multiple RMSF strains of varying pathogenicity with distinct geographic ranges, or infection with less pathogenic but antigenically-related rickettsiae that cross-react during diagnostic tests. These results may also reflect differences in detection, diagnostic, and treatment capabilities for RMSF by location. Further investigations into these clusters at a finer spatial scale are needed to determine why these differences exist.

269

SEROSURVEY OF LEPTOSPIROSIS IN PERUVIAN MILITARY PERSONNEL DEPLOYED TO HAITI

Mary K. Hinkle[1], Justin A. Green[2], Gregory J. Martin[3], Tadeusz J. Kochel[2], Eric R. Hall[2], Manuel Villaran[2], Josefina Garcia[2], Paul Scott[4], Christian T. Bautista[4], Warren B. Sateren[4], Michael Gray[5], Clinton K. Murray[1], Duane R. Hospenthal[1], Ryan C. Maves[2]

[1]Brooke Army Medical Center, San Antonio, TX, United States, [2]Naval Medical Research Center Detachment, Lima, Peru, [3]Uniformed Services University for the Health Sciences, Bethesda, MD, United States, [4]Walter Reed Army Institute of Research, Rockville, MD, United States, [5]Fort Sam Houston, San Antonio, TX, United States

Deployed military personnel are at risk for endemic infections while overseas. This ongoing, prospective observational study assesses the incidence of exposure of deployed Peruvian UN peacekeepers to selected infectious diseases, including leptospirosis. From December 2005 to July 2007, pre- and post-deployment blood samples were obtained from 809 Peruvian military personnel deployed to Haiti for 6-month peacekeeping missions. Post-deployment health surveys were completed by study participants. Blood samples were screened for leptospiral antibodies by PanBio ELISA. Positive samples (≥10 PanBio units) underwent microscopic agglutination testing (MAT) using a panel of 22 serovars of Leptospira. Of 506 participants with pre- and post-deployment samples, 63 had at least 1 sample with ELISA units ≥10, and 20 showed a change in ELISA units from <10 to ≥ 10. Among post-deployment samples from another 205 subjects, 20 had ELISA units ≥10. Of 142 samples tested by MAT, 19 samples from 11 participants were reactive. Three had confirmed leptospirosis (titer of ≥1:800, or four-fold or greater rise), 5 had probable leptospirosis (titer 1:400), and 3 were seroreactive (titer <1:400). Of these 11 participants, only 2 likely acquired leptospirosis in Haiti: 1 had a titer increase from 1:400 to 1:1600 for serovar Canicola, and 1 had a titer of 1:1600 for serovar Canicola (only post-deployment sample available). The third confirmed case had pre- and post-deployment titers of 1:800 for serovar Bratislava. All 5 of the probable and 1 of the seroreactive cases showed seropositivity prior to deployment. The most commonly reactive serovars among the probable and seroreactive cases were Bratislava and Cynopteri (3 each) and Autumnalis, Ballum, Canicola and Pyrogenes (1 each). Fever was reported in 9% of participants, and 7% were hospitalized during deployment. No cases of fever or hospitalization were reported by the confirmed or probable cases. Animal contact was reported in 20% of participants overall, though only 1 of the confirmed cases had animal contact. Serologic evidence of acute leptospirosis was present in only 0.2% of Peruvian military personnel recently returning from Haiti, despite previous reports of leptospirosis in neighboring Dominican Republic. Prior seropositivity, small population size and unknown freshwater exposure risk may explain the low incidence of acute leptospirosis in this cohort.

270

PREVALENCE OF *VIBRIO*, *AEROMONAS* AND *SALMONELLA* SPECIES IN VARIOUS SEAFOODS FROM SUPERMARKETS AND FRESH MARKETS IN BANGKOK, THAILAND

Joseph V. Woodring[1], Apichai Srijan[2], Carl Mason[3]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Armed Forces Research Institute of Medical Sciences, U.S. Army Medical Component, Thailand, Bangkok, Thailand, [3]Armed Forces Research Institute of Medical Sciences, U.S. Army Medical Component, Thailand, Bangkok, Thailand

From January to February 2008, a cross-sectional retail food study was conducted in Bangkok, Thailand to determine the prevalence of bacterial pathogens from uncooked retail seafood samples. 120 seafood samples of snapper, shrimp, oyster and blood cockles (30 of each) were collected and examined for the presence of *Salmonella*, *Aeromonas*, and *Vibrio* species. For each type of seafood, 15 samples were collected from fresh markets and 15 samples from supermarkets. *Vibrio* spp., *Salmonella* spp., or *Aeromonas* spp. were isolated from 118 of 120 samples (98%). Of the 120 total samples, 112 (93%) of the samples were positive for *Vibrio* spp., with 71 (63%) of these positive samples having more than one *Vibrio* species present. Forty-three (36%) of the 120 samples had *Aeromonas* spp. and 25 (21%) had *Salmonella* spp. Antimicrobial resistance patterns will be presented for all biotyped isolates.

271

PROFILE ANTIBIOTIC SUSCEPTIBILITY IN URINE CULTURES AND PRESENCE OF EXTENDED SPECTRUM β-LACTAMASE

Libia R. Vasquez, Laura Vasquez, Milagros Oviedo

Universidad de Los Andes, Valera, Venezuela

A retrospective study was conducted through the review of microbiological records of isolated from urine culture sent from several service clinical of private center in Valera Trujillo state, Venezuela. During the period from January 2005 to April 2008, 3454 specimenswere processed. A cross periods, 924 antibiotic sensitived test were done with NCCLS methods for the Kirby Bauer disc diffusion test. The extended spectrum β-lactamase (ESBL), existence was assayed by using double-disc synergy tests, also sensitivy test for quinolone; aminoglucoside and TMP/SXZ were practiced. There were mostly *E. coli* 757 (82%), *Proteus spp* (10.2%), *Klebsiella spp* (4.7%), *S. saprophyticus* (1.6%), observed in the urine cultures. The resistance rates of these microorganisms were determined. TMP/SXZ resistance was 59.2%; quinolone resistance 28%; and aminoglucoside resistance 13%. Totally, 78 ESBL existence were determined in isolated 430 gram negative bacillies from urine cultures. In conclusion, the results showed that *E. coli* is the first pathogen present and that quinolone resistance has emerged in bacteria isolated isolated from urine in this region of Venezuela.

272

EPIDEMIOLOGY OF RICKETTSIAL INFECTION IN IQUITOS, PERU

Alison Stewart[1], Amy Morrison[2], Brett M. Forshey[3], Ching Wei-Mei[4], Hugo Gálvez[5], Dominique Eza[2], Joel M. Montgomery[6], David E. Bentzel[3], Tadeusz Kochel[3]

[1]University of Michigan, Ann Arbor, Michigan, MI, United States, 2University of California-Davis, Davis, CA, United States, 3U.S. Naval Medical Research Center Detachment, Lima, Peru, 4Naval Medical Research Center, Bethesda, MD, United States, 5Instituto Veterinario de Investigaciones Tropicales y de Altura, Iquitos, Peru, 6Center for Disease Control and Prevention, Atlanta, GA, United States

Many rickettsial agents are pathogenic for humans and therefore pose a significant public health threat. Currently, however, there is little data to describing the disease burden of rickettsial infections in tropical regions of

the world. To help characterize the epidemiology of rickettsial agents in the Peruvian Amazon, we conducted a seroprevalence study for Spotted Fever group (SFGR) and Typhus group (TGR) rickettsia antibodies among humans and canines in Iquitos. Among 1,195 human sera assayed by group-specific ELISA, we found that 43.6% of participants had IgG antibodies against SFGR, and 10.3% had antibodies against TGR. Risk factors associated with prior infection by either group included occupation and socioeconomic status. Among 71 canines tested, nearly 75% were positive for anti-SFGR antibodies and 3% were positive for anti-TGR antibodies. One active SFGR infection among the dogs was detected with PCR. In addition, fleas and ticks were collected from the dogs and tested for rickettsial infection by PCR. Rickettsial DNA was detected in more than 50% of the arthropods tested. These results indicate that rickettsial transmission is widespread in Iquitos and potentially a significant source of human morbidity and mortality. Future studies will focus on detecting active rickettsial infections among febrile patients and thorough characterization of locally circulating rickettsial pathogens.

273

EXPRESSION, PURIFICATION AND REFOLDING OF TWO FRAGMENTS (X AND Y) OF THE OUTER MEMBRANE PROTEIN A (OMPA) FROM *RICKETTSIA RICKETTSII*

Hua-Wei Chen, Erin G. Glennon, Margarita T. Esteban, Wei-Mei Ching

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

The spotted fever group (SFG) of the genus Rickettsia includes many very closely related and cross-protective species. Rocky Mountain spotted fever (RMSF), caused by infection with *Rickettsia rickettsii*, is transmitted by the bite of infected ticks. It is the most severe form of the spotted fever group rickettsial diseases and is the most commonly fatal tick-borne disease in the United States. There have been attempts to develop a vaccine against RMSF. These vaccine preparations consisted of killed rickettsiae prepared from infected ticks, yolk sac of embryonated chicken eggs, and cell culture. All these vaccines failed to prevent infection. Therefore rapid and accurate diagnosis for timely treatment is crucial for counter measurement of SFG rickettsiae. The outer membrane proteins A (OmpA, 190 kDa) and B (OmpB, 135 kDa), are the two major immunodominant surface-exposed proteins of SFG rickettsiae. OmpA is uniquely present in the SFG and OmpB is expressed in both SFG and typhus group of rickettsiae. OmpA has been shown to interact strongly with sera from patients infected with *R. rickettsii* and other spotted fever group rickettsiae. These findings suggest that OmpA may be used as an antigen in diagnoses of spotted fever group infection. Because of its large size and many repeat regions, we cloned and expressed several fragments predicted to bear antigenic epitopes. Here, we reported the expression, purification and refolding of Fragment X and Y. Western blot analysis demonstrated that both fragments are recognized by SFG patient sera, suggesting that these OmpA fragments can be used to develop rapid sero-diagnostic assays and may even be a candidate for a Rocky Mountain spotted fever vaccine.

274

IN VITRO ANTIBACTERIAL ACTIVITY OF TIGECYCLINE BY TIME-KILL ASSAY AGAINST LINEZOLID-SUSCEPTIBLE AND -RESISTANT VANCOMYCIN-RESISTANT *ENTEROCOCCUS FAECIUM*

George A. Pankey, Deborah S. Ashcraft

Ochsner Cinic Foundation, New Orleans, LA, United States

Tigecycline (TIG) demonstrates *in vitro* bacteriostatic activity against *Enterococcus faecium* (vancomycin-susceptible and resistant). We were interested in whether the antibacterial activity persisted throughout 48 h in time-kill assay (TKA) using different concentrations of TIG and if similar activity occurs with vancomycin-resistant *E. faecium* also resistant to linezolid. During 2002-07, 20 genetically unique, U.S.A. clinical *E. faecium* isolates [10 vancomycin-resistant (VRE), 10 vancomycin and linezolid-resistant (LRVRE)] were collected. Identification was determined using the Vitek. TKA was performed following NCCLS (presently CLSI) 1999 guidelines and using TIG concentrations equal to 1, 2, and 4 times the MIC. Colony counts were determined at 0, 4, 8, 12, 24, 36, and 48 h. Fresh Mueller-Hinton II broth (<12h old) was used for all testing. Bacteriostatic activity was defined by a decrease (< 3 $\log_{10}$) in CFU/ml. TIG MICs for the VRE and LRVRE were 0.015 - 0.25 µg/ml and 0.015 - 0.06 µg/ml respectively. There is no FDA-approved breakpoint for TIG and *E. faecium.* The FDA-approved breakpoint for TIG and *E. faecalis* (vancomycin-susceptible only) is < 0.25. Bacteriostatic activity was demonstrated by all isolates with all TIG concentrations during the 48h TKA. When compared to the original inoculum, antibacterial activity increased when the TIG concentration increased. In conclusion, TKA showed that TIG demonstrated the expected *in vitro* antibacterial activity, and it persisted throughout the 48 h test period. Increasing the TIG concentration to 2x and 4x MIC maintained and increased the bacteriostatic activity. There is no data suggesting that these *in vitro* results can be translated into *in vivo* applications. Clinical data for TIG against VRE and LRVRE is needed.

275

DETECTION OF LEPTOSPIRAL DNA FROM INOCULATED BLOOD AND URINE SAMPLES USING FIVE PCR PRIMERS

Janelle L. Robertson[1], Sara J. Becker[1], Xin Yu[2], Joshua A. Hawley[1], Matthew E. Griffith[1], Miriam L. Bekius[2], Duane R. Hospenthal[1], Katrin Mende[2], Clinton K. Murray[1]

[1]Brooke Army Medical Center, San Antonio, TX, United States, [2]Infectious Disease Clinical Research Program, San Antonio, TX, United States

The current methods of diagnosing leptospirosis are unreliable. A variety of PCR primers have been developed to detect leptospires and evidence suggest that some are effective on clinical samples. We evaluated promising primers on inoculated blood and urine samples. Knowledge of how PCR performs on clinical samples is important prior to introducing the method into clinical trials. Twenty-four *Leptospira* serovars (11 species) were grown in pure culture. The DNA from these 22 pathogenic and 2 saprophytic serovars were isolated and then evaluated with PCR primers G1/G2, Lint16S, LipL32, 16s515fwd/lint16srev, and 16s641fwd/740rev. Next, the limits of detection for these 5 primers were determined using serial dilutions of *L. interrogans* serovar Porlandvere and *Leptospira interrogans* serovar Pomona in blood and urine. Dilution concentrations ranged from 10 to $1x10^6$ organisms/mL. The leptospires were counted using a Petroff-Hausser counting chamber and all 24 serovars were then added to urine and blood specimens to a concentration of $1x10^5$ organisms/mL. The primer that could differentiate saprophyte from pathogen most effectively was then used to amplify DNA from these samples. On pure culture samples, only LipL32 detected all 22 *Leptospira* serovars and did not detect either *L. biflexia* (serovars Andamana and Semoranga). 16s641fwd/740rev detected 23 of 24 isolates, including the saprophytes and 16s515fwd/lint16srev detected all 24 isolates. Lint16S detected 22 isolates, including *L. biflexia*. G1/G2 amplified 21 of the isolates, including non-pathogens. 16s641fwd/740rev had the lowest limits of detection and generated amplification products at $1x10^2$ organisms/mL. G1/G2 and Lint 16S detected $1x\ 10^3$ organisms/mL. Both LipL32 and 16s515fwd/lint16srev detected organisms at $1x10^6$ organisms/mL. While LipL32 had difficulty detecting serovars reliably in blood below $1x10^6$ organisms/mL, it was brought forward to test inoculated blood and urine samples because it performed better than the other primers in distinguishing saprophytes from pathogens. It only amplified 6 serovars in urine and 1 in blood at the clinically relevant concentration of $1x10^5$ organisms/mL. In this side-by-side comparison, the LipL32 primer was able to discriminate pathogen from non-pathogen, but underperformed on clinical samples, possibly due to the high limits of detection.

276

RISK FACTORS FOR ENDEMIC GIARDIASIS - ASSOCIATION WITH CONTAMINATED WATER AND FOOD

Johari Surin, Mohammad A. Mahdy, Yvonne A. Lim, Hesham M. Al-Mekhlafi

University of Malaya, Kuala Lumpur, Malaysia

A study to assess the risk factors of giardiasis in Orang Asli population of Pahang, Malaysia was under taken. Stool samples were collected from 321 individuals aged between 2-76 years old, of whom 160 were males and 161 females. Feacal samples were collected in screw-capped containers, fixed in polyvinyl alcohol and examined using trichrome staining technique. Biodata, personal hygiene, sanitation and socioeconomic variables were also collected via pre-tested standard questionaire. The overall prevalence rate of Giardia intestinalis infection was 22.8%. Children were significantly infected than adults. Females had higher infection rate compared to males, but the difference was statistically non-significant. Univariate analysis indicated that children less than 12 years of age are in a high risk infection group ((OR = 6.2, 9.5% CI = 1.5 - 27.0, p< 0.005). Five-fold higher risk due to giardiasis was detected among individuals drinking piped water (OR = 5.1, 95% CI = 0.06 - 0.7, p < 0.005).Individuals eating raw vegetables had a higher risk exposure than those not eating raw vegetables (OR = 2.4, 95% CI = 0.2 - 0.6, p < 0.005). A higher risk to giardiasis was also observed among individuals eating fresh fruits (OR = 2.7, 95% CI = 1.03 - 7.3, p = 0.036). Individuals in houses without toilet had higher risks than those with toilets (OR = 1.8, 95% CI = 1.1 -.3.0, p = 0.022). Logistic regression confirmed that children aged less than 12 years old; drinking piped water and eating raw vegetables are risk factors of giardiasis. On contrarily, bathing and washing clothes in the river, rearing pets, uneducated parents, working mother, house-hold members >5 and low family income are not significantly associated with giardiasis.The study showed a significant association between giardiasis and diarrhoea (Chi square = 4.574, p = 0.032) and other symptoms of gastroenteritis such as abdominal discomfort, vomiting and nausea (Chi squared = 4.832, p = 0 =0.028). Thus giardiasis still poses a great public health problem.

277

ASSOCIATION OF TNF-α PRODUCTION BY PERIPHERAL BLOOD MONONUCLEAR CELLS WITH SUSCEPTIBILITY TO AMEBIASIS

Kristine M. Peterson[1], Jianfen Shu[1], Dinesh Mondal[2], Rashidul Haque[2], William Petri[1]

[1]University of Virginia, Charlottesville, VA, United States, [2]International Centre for Diarrheal Diseases, Dhaka, Bangladesh

Entamoeba histolytica is a cause of severe dysentery and amebic liver abscess. We have followed a cohort of children from the district of Mirpur in Dhaka, Bangladesh, prospectively since 2001. *E. histolytica* infection is common here and ~90% of the children have been infected at least once. The majority remain asymptomatic, although 10% develop dysentery/ colitis. The immune response that determines whether an individual remains asymptomatic or develops disease is not well characterized. TNF-α is a pro-inflammatory cytokine which can lead to tissue inflammation, and is known to be of significant importance in the pathogenesis of inflammatory bowel disease. This study evaluated the hypothesis that the ability of PBMCs to produce TNF-α in response to amebic antigen is associated with future susceptibility to invasive amebiasis. The cohort has been intensely followed since 2001. Monthly stool samples are routinely collected from all children in the study in addition to diarrheal stools, and tested for multiple pathogens including *E. histolytica*. Whole blood was initially collected from this cohort in 2001. PBMCs were isolated prior to culture and stimulation with soluble amebic extract (SAE), and then stored at -80ºC. Cytokines including TNF-α were subsequently measured from 138 non-related children using a bioplex assay. Of the children included in this analysis, 25 had a first episode of diarrhea/colitis. Higher levels of TNF-α were associated with shorter time to first diarrheal episode (p=0.0112). With each 1000-unit increase of TNF-α, the chance to have a first episode of diarrhea was elevated by 18%. In contrast, time to first *E. histolytica* asymptomatic or total infections were not associated with TNF-α levels (p=0.3030 and p= 0.1519 respectively). After adjusting for HAZ (height for age Z-score) and WAZ (weight for age-Z score) the association with TNF-α and susceptibility to *E. histolytica* diarrhea/ dysentery remained significant (p= 0.0101 and p=0.0097 respectively). Therefore, the independent increased susceptibility seen with TNF-α was still observed even when we controlled for malnutrition. In conclusion, production of TNF-α may predict future susceptibility to *E. histolytica* diarrhea/colitis.

278

PARASITIC CO-INFECTION WITH *GIARDIA INTESTINALIS* AND *CYCLOSPORA CAYETANENSIS* AMONG CHILDREN IN PERU

Jennifer M. Ross[1], Kevin L. Winthrop[1], Dongseok Choi[1], Robert H. Gilman[2], Ynes Ortega[3], Lilia Cabrera[4], Lihua H. Xiao[5], Vitaliano A. Cama[5]

[1]Oregon Health and Science University, Portland, OR, United States, [2]Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, United States, [3]Center for Food Safety, University of Georgia, Griffin, GA, United States, [4]Asociacion Benefica PRISMA, Lima, Peru, [5]Centers for Disease Control and Prevention, Atlanta, GA, United States

Giardia intestinalis and other enteric parasites are common among children in Peru. The duration of *G. intestinalis* shedding and severity of gastrointestinal symptoms vary. It is not well known whether parasitic co-infections modulate clinical symptoms or the duration of *G. intestinalis* shedding. We collected weekly stool samples from a cohort of 485 children living in a community near Lima, Peru to identify episodes of *G. intestinalis* infection and co-infection with 20 other enteric parasites. Gastrointestinal symptoms were recorded in daily symptom surveys. The median age at entry into the cohort was 22.5 months (range 10 days to 11.5 years). Of these 485 children, 391 children (80.6%) had at least one stool sample positive for *G. intestinalis* during a median surveillance time of 31 months (range 6 months to 55 months). The frequency of *G. intestinalis* shedding ranged from never present in 94 children to present in 90.7% of samples submitted from one child. One-hundred ninety-eight children (40.8%) had at least one stool sample positive for *Cyclospora cayetanensis.* When controlling for patient age at cohort entry, *G. intestinalis* infection was not associated with *C. cayetanensis* isolation from stool (p=0.13). Diarrhea frequency was not associated with either *G. intestinalis* or *C.* cayetanensis*.* In conclusion, frequent *C. cayetanensis* infection is not an independent risk factor for frequent *G. intestinalis* infection in Peruvian children. Continuing work will correlate anti-parasitic drug treatment with the frequency of infections.

279

DEVELOPMENT OF 18S-BASED IDENTIFICATION OF *ENTAMOEBA* SPP. IN STOOL SAMPLES

Helena dos Santos[1], Kakali Bandyopadhyay[2], Rebecca Bandea[2], Regina H. Peralta[1], Jose M. Peralta[1], Mackevin Ndubuisi[3], Cindy Daniell[3], Lauren DiMiceli[3], Mahin Park[3], Alexandre J. da SIlva[4]

[1]Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, [2]Centers for Disease Control and Prevention, Division of Parasitic Diseases, NCZVED and Atlanta Research and Education Foundation, Atlanta, GA, United States, [3]Georgia Department of Health, Atlanta, GA, United States, [4]Centers for Disease Control and Prevention, Division of Parasitic Diseases, NCZVED, Atlanta, GA, United States

The genus *Entamoeba* comprises six species that can be found in human stools, i.e., *Entamoeba histolytica, Entamoeba dispar, Entamoeba moshkovskii, Entamoeba polecki, Entamoeba coli,* and *Entamoeba hartmanni*. *E. histolytica* is known to cause intestinal and extra-intestinal disease while the other species are not considered to be pathogenic,

however all species should be reported when observed in clinical samples. *E. polecki*, *E. coli*, and *E. hartmanni* can be differentiated morphologically from *E. histolytica*, but some of their diagnostic morphologic features overlap. *E. histolytica*, *E. dispar* and *Entamoeba moshkovskii* are morphologically identical but can be differentiated using molecular tools. We developed a PCR procedure followed by DNA sequencing of specific regions of 18S rRNA gene to differentiate the *Entamoeba* spp. commonly found in human stools. To date, we used this approach to analyze 8 stool specimens and 9 samples obtained from *in vitro* cultures; all were positive for *Entamoeba* spp. by microscopy and a real-time PCR method capable of differential detection of *E. histolytica* and *E. dispar*. Our results demonstrated 100% agreement between the real-time PCR for differentiation of *E. histolytica* and *E. dispar* and the 18S rRNA PCR/sequencing method. In addition, we were able to detect *E. hartmanni* and *E. dispar* in one stool sample that was previously reported as positive for *E. histolytica/E.* dispar by morphology and positive for *E. dispar* by real-time PCR. Further microscopic evaluation of this sample revealed the presence *E. hartmanni* cysts, which were undetected during the first microscopic evaluation. This approach will be useful to refine the diagnostic detection of *Entamoeba* spp. in stool and other clinical specimens.

280

PREVALENCE OF ASYMPTOMATIC *ENTAMOEBA HISTOLYTICA* INFECTION AND INTESTINAL ANTI-LECTIN IGA ANTIBODIES IN SOUTHERN INDIA

Meghan K. Rothenberger[1], Prabha Adhikari[2], William Stauffer[1], Rajeev Arvindakshan[2], Mohamed Abd-dalla[1], Ye-Ying Cen[1], Jonathan Ravdin[1]

[1]University of Minnesota, St. Paul, MN, United States, [2]Kasturba Medical College, Mangalore, India

The enteric protozoan *Entamoeba histolytica* is a significant cause of morbidity and mortality. Considering current development of an experimental amebiasis subunit vaccine, it is imperative to determine if the Gal-lectin epitopes utilized in the vaccine are conserved across multiple geographic regions and to better characterize the epidemiology of *E. histolytica* to identify populations that would benefit from vaccination. We determined the point prevalence of *E. histolytica* in urban and rural communities in Mangalore India, assessed for environmental and socioeconomic variables associated with infection, and will compare the lectin epitopes found in these subjects to those in studied populations in South Africa, Egypt, and Tanzania. Stool samples were collected from asymptomatic volunteers, frozen at -20C and shipped to the US, where they were tested for *E. histolytica* and the nonpathogenic *Entamoeba dispar* (*E. dispar*) DNA by real time PCR; ELISA was used to detect intestinal anti-lectin IgA, reflective of previous infection with *E. histolytica* or *E. dispar*. A total of 259 subjects participated (119 in urban region; 140 in rural region). Subjects ranged in age from 17 to 85 years (mean 45.2 years), the majority were female (77.2%). Of the 259 fecal samples tested by PCR, *E. histolytica* was detected in 2% and *E. dispar* in 5.9% (p=0.038). Intestinal anti-lectin IgA antibodies were detected by ELISA in 7.8% of 156 fecal samples tested. There were no socioeconomic or environmental variables including sex, age, number of inhabitants per household, literacy, employment, marriage status, water source, or latrine type that were associated with positive PCR or fecal IgA results. There was also no difference in PCR or intestinal IgA results between the urban and rural populations, although these populations differed in regards to age (50 vs 40 years, p=<0.0001), employment (36.2% vs 74.8%, p=<0.0001), marriage (63.8% vs 76.6%, p=0.025), well water use (4.3% vs 12.1%, p=0.033), and literacy (86.8% vs 76.3%, p=0.036). Gal-lectin epitope mapping studies are in progress. In conclusion, *E. histolytica* remains endemic in Southern India in both rural and urban populations; further study is needed to determine risk factors for infection and the applicability of Gal-lectin subunit vaccines to Indian populations.

281

TARGETING THE PROCESS OF ATTACHMENT IN *GIARDIA LAMBLIA* PATHOGENESIS

Colleen D. Walls, Heidi G. Elmendorf

Georgetown University, Washington, DC, United States

The intestinal parasite, *Giardia lamblia*, is a major cause of diarrheal disease and annually infects millions of people worldwide. In the U.S. the most common treatment for giardiasis is metronidazole, which has been associated with high rates of reoccurrence and reports of drug resistance. Growing concerns regarding current treatment failures have generated a need to develop novel drug alternatives. Importantly, to establish an infection *Giardia* parasites must attach to the epithelial cells lining the small intestine. To further investigate potential drug candidates, we developed a medium-throughput assay to rapidly measure *Giardia* attachment in response to the ~2,000 compounds included in the NCI diversity set small molecule library. Our primary screening at 50 μM identified 85 compounds that strongly inhibited *Giardia* attachment (≥80%), and 142 compounds that moderately inhibited attachment (79-50%). Hits generated from our primary screening represent ~11% of the NCI diversity set library. To further evaluate these compounds as potential drug candidates, we developed a viability screening using an IEC-6 rat intestinal cell line to examine drug effects on host cell viability. Results from our viability screening identified a total of 17 compounds that strongly inhibited *Giardia* attachment (≥80% inhibition), but only minimally affected IEC-6 cell viability (cell attachment and viability ≥80%). Additionally, we identified 56 compounds that moderately inhibited *Giardia* attachment (79-50% inhibition), but had only a minimal effect on IEC-6 cell viability (cell attachment and viability ≥80%). Compounds that strongly inhibited *Giardia* attachment, but only weakly affected host cell viability represent ~7% of our primary hits and ~ 0.9% of the complete NCI diversity set library. These compounds are currently undergoing further evaluation at a broader spectrum of concentrations. While drug candidates identified in our study will need further evaluation to determine their mode of action and potential toxicity, this work has provided an important first step in the discovery and development of novel drug therapies.

282

USE OF PRINCIPAL COMPONENT ANALYSIS TO EVALUATE WEALTH AND ITS ASSOCIATIONS WITH ENTERIC PARASITIC INFECTIONS IN A LOW-INCOME COMMUNITY

Vitaliano A. Cama[1], Shantanu Nundy[2], Lilia Cabrera[3], Rosa Cama[3], Robert H. Gilman[2], Lihua Xiao[4]

[1]CDC-Atlanta Research and Education Foundation, Atlanta, GA, United States, [2]Johns Hopkins University, Baltimore, MD, United States, [3]A. B. Prisma, Lima, Peru, [4]Centers for Disease Control and Prevention, Atlanta, GA, United States

We examined the relationship between wealth and parasitic enteric infections in a pediatric longitudinal study conducted from 2001 to 2006 in the community of Pampas de San Juan in Lima, Peru. *Giardia, Cryptosporidium, Cyclospora* and microsporidia were detected by microscopy examination of weekly stool samples and data on clinical manifestations was collected daily. The socioeconomic data was collected in structured questionnaires and used to construct a global wealth index using principal component analysis (PCA). It incorporated data from variables observed in >10% or <90% of study participants. Parasitological, clinical and wealth index data were analyzed using continuous and categorical models, in the latter, participants were categorized into tertiles of wealth. The analysis included data from 492 of 537 participants who had ≥6 month participation in the study, and ≥80% compliance with study procedures. The mean age was 3.43-years [0-12] and participants had a mean follow-up of 993 days. Using uni- and multivariate analyses we identified significant associations between wealth and infections with *Giardia* and microsporidia. For microsporidia, being wealthier was protective in a dose dependent fashion (p=0.066 as continuous variable,

p=0.042 by tertiles, respectively). Participants with greater wealth indexes were strongly associated with protection against *Giardia* infections (p<0.001), and persistent *Giardia* infections lasting (>14 days). Contrarily, infections with *Cryptosporidium* and *Cyclospora* were independent of the wealth categories of the participants. Our findings show that PCA models could be used for overall assessment of wealth and its impact on the prevalence of specific enteric parasites.

283

SEROPREVALENCE OF *TOXOPLASMA GONDII* IN GOATS FROM SOUTHWESTERN MISSISSIPPI

Jamela S. Alexander, Alex D. Acholonu

Alcorn State University, Alcorn State, MS, United States

Infections by the protozoan parasite, *Toxoplasma gondii*, are widely prevalent in humans and animals worldwide. The goat has gained more popularity over time and its product is being consumed by humans in the U.S. more often than ever before. The higher consumption rate of goats increases the significance of determining the problem it poses to public health in the state of Mississippi. While some studies have been done on toxoplasmosis in pigs in the state of Mississippi, there are no known studies prior to this which dealt with the prevalence of toxoplasmosis in goats in Mississippi. This study was conducted to assess the extent to which goats in Mississippi are infected with *Toxoplasma gondii*. During the period of August 2007 and April 2008, a total of one hundred and forty-four serum samples were collected from goats in four counties of Mississippi, namely, Adams (36 goats), Copiah (17), Hinds (55), and Yazoo(36). The samples were tested in three serial dilutions of 1:25 titer, 1:50, and 1:500 using the Modified Agglutination Test (MAT) method. A titer of 1:25 was considered to be seropositive. This study showed that *Toxoplasma gondii* infection is prevalent in goats from Southwestern Mississippi. It indicated that 18 (12.5%) were seropositive for *Toxoplasma* antibodies at 1:25 titer, 7(5%) at 1:50 and 2 (1.4%) at 1:500. The overall prevalence was 18(12.5%) of 144 goats, which is a cause for concern. The goat is a host record for *Toxoplasma gondii* in the state of Mississippi. A need for further studies on the prevalence of this non-specific and debilitating parasite in the state of Mississippi cannot be over emphasized.

284

COMPARISON OF MOLECULAR MARKERS FOR THE DETECTION OF VIABLE/INFECTIOUS PROZOAN PARASITES USING MOLECULAR AND CELLULAR ASSAYS

Absar Alum[1], **M. Khalid Ijaz**[2]

[1]Arizona State University, Department of Civil and Environmental Engineering, Tempe, AZ, United States, [2]Reckitt Benckiser Inc., Center of Innovation, Montvale, NJ, United States

Intestinal infections by protozoan parasites are responsible for significant part of the morbidity and mortality in developing countries and occasionally making headlines in the developed world. Conventionally, chemotherapeutic intervention has been stressed for controlling intestinal parasitic diseases around the globe. However, based on the lessons learned through global chemotherapeutic intervention programs it is can be concluded that improvement of environment and sanitation, is of paramount importance in an integrated approach for achieving desired benefits of chemotherapy. With emerging and re-emerging pathogens on the rise, the use microbicides to intervene their dissemination cannot be over emphasized. *Cryptosporidium* is among the parasites which are equally relevant to both developed and developing countries. Conventionally, non metabolic genes such as heat shock protein (hsp) and β-tubulin has been used as markers for determining the viability/infectivity using molecular techniques. In this study we have used amyloglucosidase (AG) - a metabolic protein, as marker to viability/ infectivity of *Cryptosporidium*. *Cryptosporidium* oocysts (10^3) were exposed to 6% hydrogen peroxide for 2 minutes. Samples were analyzed by cell culture polymerase chain reaction using PCR primers specific for hsp 70 and AG. Both target genes were amplified with the same level of intensity. Based on the results it can be concluded that amyloglucosidase is a valid target to study environmental survival and to evaluate the efficacy of microbicides against *Cryptosporidium* using molecular and cellular assays.

285

IBP45: A UNIQUE HOMOLOGUE OF INITIATOR BINDING PROTEIN (IBP39) IN PRIMITIVE PROTOZOAN PARASITE *TRICHOMONAS VAGINALIS*

Shweta Srivastava, Patricia J. Johnson

Department of Microbiology Immunology and Molecular Genetics, University of California Los Angeles, Los Angelss, CA, United States

The *Trichomonas vaginalis* Inr binding protein (IBP39) is a well characterized 39 kDa nuclear protein with a 14.5 kDa amino-terminal and 25 kDa carboxyl-terminal domains. The N-terminal domain binds specifically to the Inr (TCA+1Y(T/A)) and the C-terminal domain interacts with RNAP II large subunit CTD during transcription. *T. vaginalis* genome database analysis reveals presence of 7 IBP39 like genes with ≈60-40% similarity to IBP39 at the amino acid level. IBP45 is the most divergent homologue of IBP39 at the sequence level with 22% identity and 39% similarity. In this study we have purified and partially characterized IBP45 in T. vaginalis. Our preliminary data suggests that 6 out of 7 IBP homologues are expressed in T. vaginalis. We further characterized IBP45 which localizes to the nucleus thus a nuclear protein. Our results indicate that IBP45 binds to the DNA. However, it does not bind to the Inr (TCA+1Y(T/A) with the similar assays performed with IBP39. IBP45 also does not interact directly to the CTD of RNAP II. We hypothesize that IBP45 may act as a regulatory protein in transcription by binding to a unique DNA motif and take part in transcription pre initiation complex (PIC) but does not interact directly to RNAPII. This study will reveal divergent features of the basic machinery utilized to regulate gene expression in primitive protozoan parasite and its human host that might be exploited for drug design and will also provide insight into the evolution of gene regulation.

286

ANTI-PARASITIC ACTIVITIES OF THIAZOLIDES AND POTENTIAL DRUG TARGETS IN *NEOSPORA CANINUM* AND HOST CELLS

Andrew Hemphill, Joachim Müller

University of Berne, Berne, Switzerland

The nitro-thiazolides Nitazoxanide and its deacetylated derivative Tizoxanide represent broad-spectrum anti-parasitic drugs, currently used in human patients against giardiasis and cryptosporidiosis. In order to develop novel thiazolide-derivatives against parasitic diseases in food and breeding animals, the potentially harmful thiazole-associated nitro-group was modified by other functional groups. We investigated 29 different thiazolide derivatives, of which one, the bromo-thiazole-compound Rm4822 and its deacetylated metabolite Rm4847 were further studied. All thiazolides induced rapid egress of parasites out of their host cells, a process that was inhibited by the membrane-permeable calcium chelator BABTA-AM. Following egress, TIZ-treated parasites remained largely bound to the HFF host cell surface, and reinvaded host cells and resumed proliferation in the absence of the drug. In contrast, Rm4847 did not reinvade their host cells, and proliferation of parasites stopped. Rm4847 did not affect the structural integrity and viability of confluent HFF monolayers, but the proliferation of subconfluent cultures was strongly inhibited. Compounds were coupled to epoxy-agarose, and protein disulfide isomerase (NcPDI) was identified and characterized as a TIZ-binding protein in *Neospora caninum* tachyzoites, whose enzymatic activity was inhibited by a number of thiazolides, including TIZ and Rm4847. In contrast, TIZ- and Rm4847-affinity chromatography of HFF-extracts identified human quinone-reductase (QR) as a host cell protein binding to these compounds. We here present the characterization of

these putative drug targets in both parasite and host cells in relation to the putative mode of action of thiazolides against *N. caninum* and possibly other intracellular parasites.

287

EVALUATION OF STOOL FIXATIVES FOR MORPHOLOGIC AND MOLECULAR DIAGNOSIS OF CRYPTOSPORIDIOSIS

Stephanie P. Johnston, Yvonne Qvarnstrom, Michael Arrowood, Henry S. Bishop, Alexandre J. da Silva

Centers for Disease Control and Prevention, Division of Parasitic Diseases, NCZVED, Atlanta, GA, United States

Formalin and mercuric chloride low-viscosity polyvinyl alcohol (LV-PVA) are widely used in diagnostic parasitology to preserve helminth eggs, protozoan cysts, trophozoites, and oocysts in fecal specimens. Formalin is the recommended stool fixative for identification of *Cryptosporidium* spp. oocysts in stools for morphologic and immunologic methods such as acid-fast stain, direct fluorescent antibody (DFA), enzyme immunoassay (EIA), and rapid immunoassay cartridges. However, stool samples fixed in formalin produce substandard results in PCR assays. Therefore, we evaluated alternative stool fixatives that would allow molecular and microscopic detection. The following fixatives were evaluated: 4 fixatives from Meridian Bioscience, Inc. (LV-PVA, Zn-PVA, SAF, and EcoFix®), Proto-*fix*™ CLR, Alpha-Tec Systems, Inc., UNIFIX no PVA, Medical Chemical Corporation, 100% ethanol, 2.5% aqueous potassium dichromate, and unpreserved stool. Formalin (10%) was used as the gold standard to which other fixatives were compared. Parasite-free unpreserved stool was spiked with approximately 10^6 oocysts of *C. parvum* obtained from experimentally infected calves. Spiked stools were mixed with fixatives and stored at room temperature for a period of up to 3 months. Samples were analyzed using a TaqMan PCR assay targeting the 18S rRNA gene, acid-fast stain and DFA at 10 time different points, covering the 3 month period. *Cryptosporidium* sp. DNA was detected in stools fixed in EcoFix®, UNIFIX no PVA, and Zn-PVA at all 10 time points. EcoFix® and UNIFIX no PVA also allowed identification of spiked *C. parvum* oocysts on acid-fast stained smears and by DFA. Detection of *Cryptosporidium* sp. was also possible in unpreserved stools and stools preserved in ethanol and potassium dichromate by both molecular and morphologic methods. These data show that some stool fixatives are excellent alternatives to mercury or formaldehyde based fixatives permitting both microscopic and molecular detection of *Cryptosporidium* sp.

288

INTRANASAL ADMINISTRATION OF A SALMONELLA-BASED VACCINE EXPRESSING CP15 ANTIGEN CONFERS PROTECTION IN NEONATAL MICE CHALLENGED WITH *CRYPTOSPORIDIUM PARVUM*

Ace Bryan S. Cabal[1], Patricio A. Manque[2], Ana M. Lara[2], Ute Woehlbier[2], James K. Roche[3], Jesus Emmanuel A. Sevilleja[1], Andrea Rivers-Davis[3], Gregory A. Buck[2], Richard L. Guerrant[3]

[1]Enteric Diseases Study Group, National Institutes of Health, University of the Philippines-Manila, Manila, Philippines, [2]Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond, VA, United States, [3]Center for Global Health, Division of Infectious Disease and International Health, Department of Medicine, University of Virginia, Charlottesville, VA, United States

Cryptosporidium species is a highly chlorine resistant waterborne threat worldwide. Animal models are needed that mimic the effects of cryptosporidial infection in humans, and in which clinical outcomes (growth shortfalls, intensity and duration of infection) can be quantified. Ultimately, our goal is to discover and document drivers of innate and acquired immunity to prevent and/or ameliorate cryptosporidial infection and its clinical consequences. Cohorts of 7 day old mice (N=7-8) were exposed intranasally to selected cryptosporidial antigens (Cp15, Profilin, SRK), live Salmonella vector alone (pSEC) or PBS, and challenged on day 9 with 5 x 10^6 oocysts orally. Five cohorts were undisturbed and observed for growth and bacterial shedding in stool. The remaining cohorts were euthanized in 3 day-intervals post-infection for histology and assessed for intestinal colonization. Detection and quantification of *Cryptosporidium* was performed by qPCR. Sera and tissue were collected and analyzed for specific immune responses against recombinant antigens and crude parasite extract. Intranasal live-vector delivery of Cp15 and Profilin, but not SRK, significantly reduced growth short falls throughout until at least day 30 of life ($p<0.01$). Only immunization with Cp15 was able to reduce stool shedding after day 30 to negligible levels ($p<0.04$). Examination of the ileum and colon in non-immunized, but oocyst-challenged controls showed epithelial disruption (HandE staining) and parasite persistence at the mucosa, likely resulting in the observed growth shortfalls. RT-PCR analysis of intestinal tissue prior to challenge revealed differential cytokine profiles, e.g. Cp15 induced MCP-1, but not INF-γ or TNF-α, suggesting different patterns of activation of innate immune responses by our vaccinogens. Interestingly, western blot analysis indicated variant recognition of parasite derived antigens by sera from immunized and challenged versus non-immunized but challenged animals. Immunization with Cp15 leads to protection as revealed by improvement of growth shortfalls, and reduction of intensity and duration of stool shedding following *C. parvum* challenge of neonatal mice. This effect likely involves activation of innate as well as antigen-specific adaptive immune responses.

289

SIMULTANEOUS DETECTION OF *ENTAMOEBA HISTOLYTICA*, *CRYPTOSPORIDIUM PARVUM* AND *GIARDIA LAMBLIA* IN FECAL SAMPLES USING A SINGLE ENZYME IMMUNOASSAY

Cynthia Snider[1], Mamun Kabir[2], Joel Herbein[3], Jan Hencke[3], Rashidul Haque[2], William A. Petri Jr.[1]

[1]University of Virginia, Charlottesville, VA, United States, [2]International Centre for Diarrheal Disease Research- Bangladesh, Dhaka, Bangladesh, [3]TechLab, Inc., Blacksburg, VA, United States

A fecal screening test is needed to detect the three most common enteric protozoan parasites in the U.S. Microscopic ova and parasite (OandP) exam of stool suffers from poor sensitivity and specificity. The detection of parasite antigen in stool by enzyme-linked immunoassay (ELISA) is the current diagnostic method of choice in comparison to real-time PCR tests (RT-PCR) which is not yet cost-effective as screening assay. The purpose of this study is to evaluate the Tri-Combo ELISA performance characteristics of simultaneously detecting *Entamoeba histolytica, Cryptosporidium, and Giardia lamblia* in stool samples against the gold standard of individual specific ELISA and RT-PCR in an endemic setting. Fecal specimens were obtained from children and adults in Dhaka, Bangladesh. The Tri-Combo is a conventional two-step ELISA format with HRP-conjugated detecting antibodies for colorimetric development in a single assay well for stool samples with either *Giardia, Cryptosporidium,* or *E.histolytica.* For those samples with discrepancy results between the Tri-Combo versus individual specific antigen test, RT-PCR analysis was conducted. A total of 235 stool specimens tested. Of the 97 samples with parasitic infection: 59 (61%) *Giardia*, 25 (26%) *Cryptosporidium*, and 13 (13%) *E.histolytica*. The Tri-Combo ELISA has 99% sensitivity and 96% specificity, 95% positive predictive value and 99.2% negative predictive value. The Tri-Combo has a 97% correlation to individual parasite ELISA. In conclusion, this preliminary data of the Tri-Combo ELISA for simultaneous detection of *Giardia*, *Cryptosporidium* and *E. histolytica* reveals similar test characteristics as individual parasite ELISAs. This diagnostic screening test represents a potential cost savings in the detection of enteric parasite infections, especially in resource constraint settings.

290

CASPASE 9, A SIGNALING PROTEIN OF THE HUMAN LIVER FLUKE, *OPISTHORCHIS VIVERRINI*

Sandi K. Parriott[1], Thewarach Laha[2], Banchob Sripa[3], Alex Loukas[4], Paul J. Brindley[1]

[1]Department of Microbiology, Immunology and Tropical Medicine, The George Washington University, Washington, DC, United States, [2]Department of Parasitology, Khon Kaen University, Khon Kaen, Thailand, [3]The Department of Pathology, Khon Kaen University, Khon Kaen, Thailand, [4]Division of Infectious Diseases and Immunology, Queensland Institute of Medical Research, Brisbane, Queensland, Australia

Cholangiocarcinoma (CCA) - cancer of the bile ducts - is associated with chronic infection with the oriental liver fluke, *Opisthorchis viverrini*. Despite being the only eukaryotic organism that is designated as a 'class I carcinogen' by the International Agency of Research on Cancer (IARC), little is known about the transcriptome and genome of this enigmatic parasite. A gene discovery project for *O. viverrini* using the expressed sequence tag (EST) approach was begun in 2007, as reported previously. Among other genes, ESTs representing putatively secreted or transmembrane proteins with known roles in tumor induction and progression were identified, and these might play roles in the pathogenesis of *O. viverrini*-induced CCA. The preliminary characterization of one of these secreted or transmembrane proteins, an orthologue of caspase 9, is underway. Caspase 9 is the apical caspase of the intrinsic pathway of apoptosis which is activated by the release of cytochrome c from the mitochondrion by way of interaction with the apoptosome. It activates the executioner caspases 3 and 7 in response to ionizing radiation, chemotherapeutic drugs, and certain developmental cues. The open reading frame (ORF) of the *O. viverrini* caspase 9 orthologue encodes a protease of 372 amino acid (aa) residues, with a NH2-terminal caspase recruitment domain (CARD) and COOH-terminal caspase IL-1β converting enzyme domain (CRSc). Blast analysis revealed that its closest relatives were caspase 9 from *Gallus gallus* and *Xenopus laevis*. Recombinant fusion proteins of *O. viverrini* caspase 9 have been produced in *Escherichia coli* and purified by affinity chromatography. This protein is likely a component of a crucial pathway involved in cell death and may be associated with pathogenesis of cholangiocarcinoma in *O. viverrini*-infected individuals. Ongoing and future research includes isolation of recombinant caspase 9 away from the NusA fusion partner, optimization of affinity purification of soluble forms of the protease, enzyme activity assays, and polyclonal antibody production. We plan to investigate immunolocalization of this signaling protein within developmental stages of the fluke, the fluke's biochemistry and cell biology, and its interactions with cultured human biliary cell lines.

291

TEMPERATURE-INDUCED GENE EXPRESSION OF *CLONORCHIS SINENSIS* NEWLY EXCYSTED JUVENILE IN ADAPTATION TO MAMMALIAN HOST

Won Gi Yoo[1], Tae Im Kim[1], Shunyu Li[1], Pyo Yun Cho[2], Tong-Soo Kim[3], Sung-Jong Hong[1]

[1]Chung-Ang University, Seoul, Republic of Korea, [2]Centers for Disease Control and Prevention, Seoul, Republic of Korea, [3]In-Ha University, In-Cheon, Republic of Korea

When ingested, *Clonorchis sinensis* newly excysted juveniles (CsNEJs) encounter heat shock in mammalian hosts. CsNEJs acclimate to 37°C and develop to adult worms. This study was performed to analyze global gene expression of CsNEJs during thermal adaptation using oligonucleotide microarrays. The microarray was fabricated with 11,486 probes designed on *C. sinensis* expressed sequence tags. CsNEJs were incubated at 37°C for 6 hours. The 705 up- and 139 down-regulated genes showing 2-fold or greater differences were considered as temperature-induced ones. Heat shock enhanced the transcription of metabolic processes, i.e. energy and amino acid metabolism, signal transduction, transcription and translation. Many other genes were also up-regulated, such as heat shock proteins (40, 70, 105 kDa), channels and transporters, structure proteins, protein kinases and phosphatases. Protein kinases and phosphatases regulate basic functions such as DNA replication, cell cycle and protein synthesis. Oxalate/formate antiporter may be responsible for increased energy production. Down-regulated genes were of heat shock 49 kDa, ribonucleoprotein, calpain, collagen type III α 1 protein. Ribonucleoprotein associated with RNA splicing may be related with increased protein synthesis. To understand biological networks, pathways involved in energy metabolism and amino acid metabolism were redrawn in Kyoto Encyclopedia of Genes and Genomes database. In conclusion, HSPs were expressed in a developmental stage-specific pattern. This analysis on gene expression and metabolic pathway provides valuable insight into biological features of CsNEJs in adaptation to 37°C.

292

CHOLIC ACID AND DOPAMINE NEURONS DRIVE BILE-CHEMOTAXIS OF *CLONORCHIS SINENSIS* NEWLY EXCYSTED JUVENILE

Shunyu Li[1], Tae Im Kim[1], Won Gi Yoo[1], Pyo Yun Cho[2], Tong-Soo Kim[3], Sung-Jong Hong[1]

[1]Chung-Ang, Seoul, Republic of Korea, [2]Centers for Disease Control and Prevention, Seoul, Republic of Korea, [3]In-Ha University, In-Cheon, Republic of Korea

Neuro-receptors associated with bile-chemotactic behavior of *Clonorchis sinensis* newly excysted juveniles (CsNEJs) were analyzed by neuropeptides and inhibitors of glutamate, serotonin, dopamine and acetylcholine neuron groups. The CsNEJs migrated toward 0.001-0.01% bile but away from 0.05-0.1% bile. The CsNEJs showed strong chemotactic behavior to cholic acid, but chemorepellent to lithocholic acid. The bile chemotaxis was inhibited strong by dopamine receptor antagonists D1 (SKF 83566 and LE-300), D2 (AMI-193, Nemonapride and RS-(+/-)-sulpiride), D3 (GR 10369 and NGB) and dopamine uptake inhibitor (BTCP), but showed chemo-repellent to 1 and 10 μM of AMI-193 and NGB. The bile chemotactic migration was inhibited by serotonin, glutamate and acetylcholine receptor inhibitors at high concentration. Collectively, chemotaxis of CsNEJs toward cholic acid is considered to be a driving factor of migration to the bile ducts in the final hosts. It is proposed that dopamine neurons play a major role of bile-chemotactic behavior of *C. sinensis*, and serotonin and acetylcholine neurons may take minor parts for the chemotactic responses.

293

PROPHYLACTIC EFFECT OF ARTESUNATE WITH/WITHOUT ANTIOXIDANTS ON JUVENILE AND ADULT EGYPTIAN STRAIN OF *SCHISTOSOMA MANSONI* IN MICE

Sayed H. Seif el-Din

Theodor Bilharz Research Institute (TBRI), Giza, Egypt

Artesunate (Arts), a derivative of the antimalarial artemisinin, also exhibit antischistosomal properties. The antioxidants vitamin C (vC) and selenium (Se) protect hepatocytes from undergoing apoptosis, attenuate experimental liver fibrosis and can improve survival. This study investigates the therapeutic potential of artesunate in *Schistosoma mansoni*-Egyptian strain when used alone or in addition to vC and Se and to evaluate the protective role of these antioxidants on *S. mansoni*-induced oxidative stress. Mice were divided into 7 groups; normal (i), infected control (ii), infected+vC (10 mg/kg/day) and Se (500 mcg/kg/day) in drinking water (iii), infected treated with Arts (300 mg/kg) at juvenile (iv) and adult (v) *S. mansoni*, and infected+(vC-Se) and treated with Arts at juvenile (vi) and adult (vii) *S. mansoni*. Supplements with vC and Se started from the 1st day of infection until sacrifice 10 weeks post infection. Results showed that Arts reduced significantly both female and total number of worms when used alone at adult and juvenile stages of infection. Also, tissue egg loads were decreased accompanied with an increase in the

percentage of dead ova. Supplementation with vC and Se alone increased the percentage of dead ova, meanwhile, it enhanced the decrease in female, total number of worms, tissue egg loads and produced a complete disappearance in the percentage of total immature ova stage when used with Arts at juvenile stage. Infection with *S. mansoni* increased tissue MDA, GR and serum ALT and GGT, while decreased the activities of SOD, GPx, GST and the contents of GSH as well as serum proteins and albumin compared to normal untreated control. Supplementation with vC and Se alone approximately recovered the contents of GSH, the activities of GPx and GST and the levels of serum albumin relative to normal control. Normalization in the activities of the antioxidant enzymes mentioned above and serum ALT, GGT, total proteins and albumin was observed in groups of mice treated with Arts alone especially at the early stage of infection or when used with vC and Se supplements. This study demonstrated that artesunate is efficient drug against immature *S. mansoni*-Egyptian strain. The use of vC and Se alone or in addition to artesunate could protect the liver tissue against *S. mansoni*-induced oxidative stress probably by increasing antioxidative defense activities and enhancing the improvement of serum markers enzymes related to liver functions.

294

EVALUATION OF SURFACE ANTIGENS OF *SCHISTOSOMA MANSONI* AS VACCINE CANDIDATES

Jayendra Prasad, Erica Waite, Shifan Liu, Ronald E. Blanton, Christopher L. King

Case Western Reserve University, Cleveland, OH, United States

Efforts to produce an effective vaccine against schistosomes have often used an empiric approach that focuses on one or a small number of antigens. Vaccination experiments with irradiated cercariae all indicate that the early to lung stage schistosomula are most susceptible to immune attack. It is likely that antigens exposed to the human blood stream are the targets of this protective response. In developing a new approach to target antigen discovery, proteomics was used to identify a set of antigens localized to schistosomal surface or excretory-secretory (ES) proteins. A total of 33 tegumental or ES proteins were identified by mass spectroscopy. These included proteases, protease inhibitors, immunoregulator homologs and structural proteins and proteins of unknown function. We have cloned 11 of the target mRNAs by amplifying from a total cDNA pool. These include annexin and venom allergen homologs, proteases, protease inhibitors and genes of unknown function. Sequencing was performed to confirm the presence of the correct ORF. The proteins were expressed with a his tag in E. coli using the T7 expression system. Nine of the proteins were expressed and purified by nickel column-chromatography. For the first round of protection studies, annexin1, smrp22 and membrane serpin were selected. For each antigen, 10 C57Bl6 mice were injected X 3 with CpG adjuvant and challenged with 120 cercariae. A control group was also immunized with a purified malarial recombinant protein. All antigens stimulated high antibody titers. The animals were sacrificed after 6 weeks, and adult worms perfused. While Annexin1 gave ~18%, membrane serpin gave ~30% less worms relative to the control. Interestingly, Smrp22 gave a higher number of worms than the control. This suggests that Smrp22, a protease of the subtilisin family, may modulate the immune response to infection by *S. mansoni*. We are in the process of cloning and testing the other targets as well as expressing these in the baculovirus system to check whether posttranslational modifications play a role in protection.

295

HEALTH TECHNOLOGY ASSESSMENT OF *SCHISTOSOMA MEKONGI* CONTROL PROGRAM, CAMBODIA

Dysoley Lek

National Center for Parasitology, Entomology and Malaria Program, Phnom Penh, Cambodia

Schistosomiasis is one of the most prevalent parasitic infections in the world and WHO recommends large scale preventive chemotherapy as control strategy. This control intervention has been applied in Cambodia for nine years in the two provinces endemic for the disease (population at risk approximately 80,000 individuals) and resulted in the elimination of mortality and severe morbidity. The present study evaluated the cost of the implementation and maintenance of the *Schistosomiasis* control programme applying a "full costing approach" that takes into consideration not only the direct costs but also several other costs that are not considered in other studies available in the literature. The programme has been divided into an implementation phase and a maintenance phase. During the 9 years intervention the prevalence of the disease decreased from 80% to less than 1%. No more cases are identified during the survey and no morbidity and mortality due to schistosomiasis is reported. During the maintenance phase large scale preventive chemotherapy is applied to maintain the low level of prevalence obtained. The total cost of the 9 years implementation phase of the programme (8 drug distributions) has been valued at 591,662 USD; the cost for each averted death has been evaluated at 5,060 USD, the cost for a severe infection avoided at 48 USD and the cost for each infection avoided at 9.59 USD. The cost for an averted death is in the range of the one obtained by other interventions that are considered extremely cost-effective in developing countries, like the measles vaccination or the distribution of insecticide-impregnated bed nets used for malaria control. In addition to the elimination of the morbidity and mortality due to the programme produced an average increase in productivity evaluated at over 5 million USD. We estimated that the return for each dollar invested in the *Schistosomiasis* control programme is over 7.5 USD. Despite the very good Cost-effectiveness obtained, the cost of the programme per beneficiary (0.9 USD) represents a relevant part of the pro-capita expenditure on health from the Cambodian Government (6 USD); it is therefore very unlikely that the MoH could maintain the programme only with national financial resources, hence, the necessity for the donor community to invest in this highly efficient programme.

296

HIGH GENETIC DIVERSITY IN *SCHISTOSOMA MANSONI* IN THE SENEGAL RIVER BASIN, A POPULATION GENETIC ANALYSIS 20 YEARS AFTER THE EPIDEMIC OUTBREAK

Tine Huyse[1], Gregory E. Maes[2], Sarah Geldof[2], Kim Vereecken[1], Djibril Djibril[1], David Rollinson[3], Bonnie L. Webster[3], Katja Polman[1]

[1]Institute of Tropical Medicine, Antwerp, Belgium, [2]Katholieke Universiteit Leuven, Leuven, Belgium, [3]The Natural History Museum, London, United Kingdom

About twenty years ago, two dams were constructed in the Senegal River Basin (SRB) in order to improve the agricultural conditions in Northern Senegal. The subsequent ecological changes stimulated the growth and spreading of *Bulinus* and *Biomphalaria* snail species, intermediate hosts of *Schistosoma haematobium* and *S. mansoni*, respectively. This resulted in a major outbreak of intestinal schistosomiasis. A recent study highlighted an increase in urinary schistosomiasis while infection intensity is decreasing for *S. mansoni* (Polman K, pers. comm.). This is a unique system where the origin of an epidemic outbreak is exactly known and its evolution carefully monitored, providing an opportunity to study the molecular evolution of pathogens in a relative short time frame. Demographic parameters such as the effective population size reflects the adaptive potential of a parasite, i.e. how the parasite population can cope with selection pressure of the host or the environment, while gene flow estimates illustrate

the transmission dynamics between populations and localities. Here we report on the population genetic structure of *S. mansoni.* In March 2006 and 2007, urine and stool samples were collected along the lower and middle valley of the SRB. Eggs have been isolated and hatched. Individual miracidia were collected on Whatman FTA® indicator cards and genotyped for 9 microsatellite loci (a single multiplex). A selection of samples has also been sequenced for ITS1 rDNA and partial cox1 mtDNA. A thorough population genetic analysis revealed a high genetic diversity within populations, but low genetic differentiation between populations, suggesting extensive migration between villages.

297

MECHANISM OF ANEMIA IN *SCHISTOSOMA MANSONI*-INFECTED SCHOOL CHILDREN IN WESTERN KENYA

Sara E. Butler[1], Erick M. Muok[2], Susan P. Montgomery[1], Pauline M. Mwinzi[2], Diana M. Karanja[2], W. Evan Secor[1]

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

Anemia is associated with *Schistosoma mansoni* infection but the causal mechanism is not known. Better understanding of the mechanism of anemia associated with *S. mansoni* infection may instruct how treatment programs are implemented to minimize schistosomiasis-associated morbidity. Possible mechanisms of anemia caused by schistosomiasis include iron deficiency anemia, anemia of inflammation or a combination mechanism. In a cross-sectional study, sera from 168 school children (9-12 years old) that had screened negative for malaria and soil transmitted helminth infections were randomly selected and grouped according to their *S. mansoni* infection status as defined by stool exam and serology. Approximately half of the children in both groups were anemic according to the Kenya National Clinical Guidelines (hemoglobin level < 12 g/dl). To elucidate the mechanisms involved in schistosomiasis-associated anemia, serum ferritin and soluble transferrin receptor (sTfR) levels were measured by ELISA. Preliminary results suggest that children with *S. mansoni* infection were 3.84 times more likely (95% CI, 1.04 to 14.18) to have levels of serum ferritin that are associated with anemia of inflammation (> 100 ng/ml) than *S. mansoni* negative individuals. In contrast, anemic *S. mansoni* negative children had significantly higher levels of sTfR ($p < 0.01$) than the group with normal hemoglobin levels, suggesting iron deficiency anemia, while *S. mansoni*-infected individuals' sTfR levels did not significantly differ in the anemic and nonanemic subgroups. These findings are consistent with *S. mansoni* infection increasing the likelihood of anemia of inflammation in school age children, similar to what has also been observed in studies of *S. japonicum*-infected individuals.

298

POSSIBLE PRESENCE OF THREE PROMINENT IMMUNE SIGNALING PATHWAYS, IMD/RELISH, TOLL/DORSAL AND JAK/STAT, IN THE SNAIL *BIOMPHALARIA GLABRATA*, THE INTERMEDIATE HOST OF *SCHISTOSOMA MANSONI*

Si-Ming Zhang, Vijay Ramakrishnan, Hong Nian

University of New Mexico, Albuquerque, NM, United States

Recent studies suggest that IMD/Relish, TOLL/Dorsal, and JAK/Stat, the evolutionarily conserved signaling pathways, play critical role in regulation of immune responses to various pathogens in vertebrates and invertebrates. However, little is known about the existence of the pathways and their associated components, in the snail *Biomphalaria glabrata*, the intermediate host of human parasite *Schistosoma mansoni*. In order to better understand the snail's internal defence mechanisms, we have characterized three transcription factor genes, *BgRelish*, *BgDorsal*, and *BgStat* from the Bge cell line of *B. glabrata*. BgRelish, BgDorsal, and BgStat are homologues of Relish, Dorsal/Dif, and Stat transcription factors respectively in arthropods. It has been shown that Relish, Dorsal/Dif, and Stat transcription factors are involved in control of IMD, TOLL, and JAK pathways, respectively. The sequence analysis revealed that *BgRelish* encodes a 1110-aa class 1 Rel-like protein, which possesses a RHD (Rel homology domain), an IPT (Ig-like, plexins, transcription factor), 6 ankyrin repeats, and a death domain at C terminus. *BgDorsal* encodes a 610-aa class 2 Rel-like protein consisting of RHD, IPT, and a C-terminal transactivator domain (TD). The amino acid identity of RHD between the two snail NF*k*B homologues (BgDorsal and BgRelish) is about 40%. Additionally, *BgStat*, another transcription factor gene, encoding a 759-aa protein has also been identified. Like most Stat proteins, BgStat is composed of an interaction domain, an all-α domain, a DNA binding domain, and a Src homology 2 (SH2) domain. Our primary data suggest that the three most prominent immune signaling pathways exist in *B. glabrata*. Functional characterization of the three transcription factors and their associated pathways are currently under investigation.

299

COMMUNITY-DIRECTED INTERVENTION FOR SCHISTOSOMIASIS AND SOIL TRANSMITTED HELMINTHES IN WESTERN KENYA

Pauline NM Mwinzi[1], Mariam Mwanje[2], Chrispin Owaga[1], Erick Muok[1], Kayla Laserson[3], Adazu Kubaje[3], Susan Montgomery[4], W. Evan Secor[4], Erick Muchiri[2], Diana MS Karanja[1]

[1]Kenya Medical Research Institute, Center for Global Health Research, Kisumu, Kenya, [2]Division of Vector Borne Diseases, Kenya Ministry of Health, Nairobi, Kenya, [3]KEMRI-Centers for Disease Control and Prevention, Kenya Medical Research Institute, Center for Global Health Research, Kisumu, Kenya, [4]Centers for Disease Control and Prevention, Division of Parasitic Diseases, Atlanta, GA, United States

Schistosome and soil-transmitted helminth (STH) infections are recognized as major global public health problems, causing severe and subtle morbidity, including significant educational and nutritional effects in children. World Health Assembly resolution 54.19 (WHA 54.19) calls for interventions to reduce morbidity from schistosomiasis and STH infections. Although effective and safe drugs are available, ensuring access to these drugs by all those at risk of schistosomiasis and STHs is still a big challenge. Community Directed Intervention (CDI) has been used for mass distribution of drugs for onchocerciasis and lymphatic filariasis. We evaluated whether CDI increased access to treatment in a community at high risk of infections with schistosomes and STHs in East Uyoma, Western Kenya. Praziquantel and albendazole were used for the integrated intervention of schistosomes and STH infections, respectively, among community members above the age of 4. Pre-treatment prevalence of *Schistosoma mansoni*, STHs and anemia were measured in class 4 school childern (9-10 year olds) in all 11 schools in the area. Pre-treatment prevalence of *S. mansoni* ranged from 5-43% in the 11 schools, and averaged 17.4% in the entire location. Trained Community Drug Distributors (CDD) delivered the medications. Treatment coverage based on CDD records ranged between 74 and 97%. Coverage from a household, perhaps more accurate, survey showed coverage of 65 to 96%. Lower coverage in some villages was attributable to the electioneering period and famine that led to absenteesm from home compounds. Focus group discussions and indepth interviews showed that the exercise was generally very well received in the community. All CDDs said they would participate in a similar exercise again. Future CDI in the country should involve appropriate timing to coincide with harvest and use of appropriate community engagement strategies in areas where the traditional village barazas are no longer the mode of conveying information. This study shows that CDI may increase treatment access and coverage.

300

CIRCULATING CYTOKINES, THEIR SOLUBLE RECEPTORS AND HUMAN RESPONSES TO PRAZIQUANTAL TREATMENT OF SCHISTOSOMIASIS

Jenny Houghton[1], Colin M. Fitzsimmons[1], Narcis B. Kabatereine[2], Gachuhi Kimani[3], Eric Muchiri[4], Joseph K. Mwatha[3], Claus M. Reimert[5], Edridah M. Tukahebwa[2], Birgitte J. Vennervald[5], David W. Dunne[1]

[1]Department of Pathology, Cambridge University, Cambridge, United Kingdom, [2]Vector Control Division, Ministry of Health, Kampala, Uganda, [3]Kenya Medical Research Institute, Nairobi, Kenya, [4]Division of Vector Borne Diseases, Kenyan Ministry of Health, Nairobi, Kenya, [5]DBL - Centre for Health Research and Development, Copenhagen, Denmark

Schistosomes live for many years in the human bloodstream very effectively evading and regulating host immune responses. Praziquantal is a highly effective treatment, but rapid reinfection, particularly among younger children, is a major public health concern. Older individuals however develop an important partial immunity to reinfection, which is associated with eosinophilia and anti-worm Th2-like responses: IL-4, IL-5, IL-13, IgE production. Treatment itself boosts these responses, probably due to the rapid release of worm antigens directly into the bloodstream. Despite this, treatment does not result in systemic hypersensitivity reactions. This association of post-treatment Th2-like responses with immunity, and the apparent tight regulation of adverse Th2-like reactions, led us to examine immediate post-treatment responses in Ugandan schistosomiasis mansoni populations. Treatment induced several changes in circulating cytokines. In particular, a preliminary study suggested that a boost in plasma IL-5 levels in some individuals 24 hours after treatment may be associated with immunity to subsequent reinfection. Cytokines themselves can be regulated by soluble cytokine receptors that can variously antagonise, agonise, or both, the effector function of the associated cytokine. Again, preliminary investigations showed changes in the circulating levels of soluble cytokine receptors after praziquantal treatment. We have now developed multiplex bead-based assays for cytokines and soluble receptors, which allow numerous analytes to be measured simultaneously in a few microlitres of sample. This, combined with fingerprick blood sampling, has allowed larger study cohorts to be sampled at more time-points. A cohort from a Ugandan fishing community was treated and finger-pick samples taken before treatment and 24 hours post-treatment. By multiplex assay, these samples were analysed for IL-4, IL-5, IL-6, IL-10, IL-13 and their soluble receptors. This data was analysed in the context of a large database of information on the cohort, covering infection levels in the 2 years following treatment plus other clinical, behavioural, demographic, immunological and geographical data. This provides insights into the role of circulating cytokines and their soluble receptors in the development of human immunity and immune regulation in response to schistosomiasis mansoni.

301

TRANSPOSITION EXCISION ACTIVITIES OF THE *PIGGYBAC* AND *MOS-1* MARINER TRANSPOSONS IN *SCHISTOSOMA MANSONI*

Yousef N. Alrefaei[1], Maria Morales[2], Paul J. Brindley[1]

[1]The George Washington University, Washington, DC, United States, [2]Tulane University, New Orleans, LA, United States

Functional genomics tools are needed to determine the importance of schistosome genes as new intervention targets. Transposable element excision assays are useful indicators of an element's ability to be mobilized *in vivo* and, thus, potentially serve as a transforming vector. We have shown recently that both the transposons *piggyBac* and *Mos1 mariner* are transpositionally active in tissues of cultured developmental stages of *Schistosoma mansoni*, including schistosomules and mixed sex adults. In addition, for *piggyBac* we observed that the transposon transgenes integrate into schistosome chromosomes and are trascriptionally active. In the present study, we have examined the precision of excision of the transposon form donor plasmid backbones after introduction into schistosomules along with mRNA encoding the cognate transposase, by square wave electroporation. Contrary to the situation reported in several other target species, excision of *piggyBac* was not precise. Donor cleavage of the piggyBac constructs occurred at a different sequences from the standard TTAA sequence. In particular, of nine excision repaired plasmids sequenced, eight included part of the inverted terminal repeat (ITR), ranging in length from 19 - 64 bp. In like fashion, excision of *Mos1 mariner* also was not precise: of eight excised plasmids recovered from transposon transformed schistosomules, three included parts of the ITR and four revealed excision of plasmid backbone sequences adjacent to the ITR. Nonetheless, the imprecise excision may not have disrupted the reporter transgene within the donor cassette. We are investigating reporter gene activity and integration of *Mos1* into the chromosomes of schistosomes, and its potential for vertical transmission to schistosome progeny.

302

A PRINCIPAL COMPONENTS ANALYSIS OF IMMUNE PARAMETERS ASSOCIATED WITH RESISTANCE TO REINFECTION WITH *SCHISTOSOMA MANSONI*

Carla L. Black[1], Pauline N. Mwinzi[2], W. Evan Secor[3], Diana M. Karanja[2], Daniel G. Colley[1]

[1]University of Georgia, Athens, GA, United States, [2]Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States

In a longitudinal study of adult males occupationally exposed to schistosomes by washing cars in Lake Victoria, we have utilized principal components analysis (PCA) to identify patterns of cytokine and antibody responsiveness and their relationship with resistance to reinfection with *Schistosoma mansoni*. The PCA included variables representing production of the cytokines IL-5, IL-10, IL-13 and IFN-γ in response to soluble worm antigen preparation (SWAP) and SWAP-specific levels of the antibody isotypes IgE, IgG1, IgG3, and IgG4. The number of reinfections per 100 cars washed in a defined time period was used as an index of susceptibility or resistance (IoSR). Increased resistance was significantly correlated with the score for principal component 6 (PC6) ($r = -0.389$, $p = 0.0451$), characterized by high positive factor loadings for IFN-γ and IgE and high negative loadings for IL-10. Increased susceptibility correlated with PC4 ($r = 0.407$, $p = 0.0353$), characterized by high positive loadings for IgG1 and IgG3 and a high negative loading for IgE. While not statistically significant, susceptibility also correlated with PC3 ($r = 0.363$, $p = 0.0627$), characterized by high positive loadings for IgE and IgG3 and high negative loadings for IL-10 and IFN-γ, and PC5 ($r = 0.326$, $p = 0.0973$), characterized by high positive loadings for IL-10 and IgG3 and a high negative loading for IL-13. Thus, the combination of high IgE and low IL-10 was associated with increased resistance in the presence of high IFN-γ and with decreased resistance in the presence of high IgG3 and low IFN-γ. In individual correlation analyses, increased susceptibility was correlated with increased anti-SWAP IgG3 production ($r = 0.441$, $p = 0.0212$). There were no correlations between IoSR and production of any other studied cytokines or antibody isotypes. These results suggest that development of resistance to *S. mansoni* reinfection is associated with a combination of immune responses that may not be evident when each immune parameter is analyzed independently.

303

CHARACTERIZATION OF HUMORAL AND CD4+ T CELL RESPONSES TO SMCB1 IN SCHISTOSOMIASIS PATIENTS RESIDING IN ENDEMIC AREAS IN BRAZIL

Lucia A.O. Fraga[1], Erika Lamb[2], Elizabeth C. Moreno[3], Luiz Cosme C. Malaquias[4], Alda Maria S. Silveira[5], Jan Dvorak[6], Conor R. Caffrey[7], Stephen J. Davies[8]

[1]Uniformed Services University of the Health Sciences/UNIVALE/DRS, Bethesda, MD, United States, [2]Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [3]Funasa-Fundação Nacional de Saúde-MS-Brasil, Belo Horizonte, Brazil, [4]UNIVALE-Universidade Vale do Rio Doce, Gov. Valadares, MG., Brazil, [5]UNIVALE-Universidade Vale do Rio Doce, Gov. Valadares, MG., Brazil, [6]Sandler Center for Basic Research in Parasitic Diseases, California Institute for Quantitative Biosciences (QB[3]), University of California, San Francisco, CA, United States, [7]Sandler Center for Basic Research in Parasitic Diseases, California Institute for Quantitative Biosciences (QB[3]), University of California, San Francisco, CA, United States, [8]Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda, MD, United States

The major objective of our study is to examine whether the human immnune response to schistosome infection mirrors the emerging picture of immune responses in the laboratory mouse model, particularly with regard to the induction of immunoregulatory T cell responses during the establishment of infection. In mice, our data show that schistosome gut proteases Sm31/SmCB1 and Sm32/SmAE are the dominant antigens recognized by the early regulatory T cell response during pre-patent infection, to be reported.. If our efforts are to advance the development of efficacious vaccines for humans, it will be critical to establish that the human immune system mounts regulatory T cell responses to the same antigens. An initial subset of patients was selected from two areas endemic for schistosomiasis, near Gov. Valadares, Minas Gerais State, in southeast Brazil. Patients were classified as (1) endemic normals, exposed to infection but with not history of schistosome eggs in their feces between 1997-2007; (2) susceptible, with previous history of or current evidence of schistosome eggs in feces; and (3) resistant or cured, with previous history of infection but without evidence of re-infection after treatment during the same period of time. Humoral and cell-mediated immune responses to crude schistosome worm antigen (SWAP) and recombinant SmCB1 were analyzed to determine whether evidence of regulatory responses to these antigens could be detected. For antibody isotype responses, patient sera archived over the past 10 years at Univale were analyzed for the presence of SWAP- and SmCB1-specific IgG1, IgG4, IgE and IgM immunoglobulins. For cellular responses, $CD4^+$ T cells and $CD14^+$ T cells (APC) were purified from PBMC by magnetic cell sorting, co-cultured at a ratio of 10:1, respectively, and stimulated with SWAP, SmCB1 or anti-CD3. Following incubation for 144 h at 37 °C in 5 % CO2, supernatants were collected and cytokine concentrations (IFN-γ, IL-10, IL-4) determined by ELISA. After overnight culture in fresh medium and stimulation with leukocyte activation cocktail and GolgiPlug (BD Pharmingen), cytokine production was also assessed by intracellular cytokine staining, cells were then analyzed by flow cytometry. Associations between all immunological variables and patient infection status (endemic normal (----), susceptible (- + - +) and resistant (- + - -)) were analyzed.

305

URINARY SCHISTOSOMIASIS SCOURGE AMONG RURAL SCHOOL CHILDREN IN CHITONGO AREA, SOUTHERN ZAMBIA

Sandra Chishimba[1], Aniset Kamanga[1], Jay Sikalima[1], Julie Clennon[2], Sungano Mharakurwa[1], Clive J. Shiff[2]

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

Urinary schistosomiasis, due to the helminth *Schistosoma haematobium*, is one of the most common parasitic diseases of children and adolescents living in rural areas of Zambia. Long-term consequences of bilharzia include haematuria, lesions of the bladder, kidney failure and elevated risk of bladder cancer. The present study discusses the magnitude of the scourge in relation to local contributory geographical aspects at three primary schools in Chitongo area of Southern Zambia. Urine samples were collected from 303 pupils (149 males and 154 females) aged 4 - 17 years old to determine the prevalence of schistosomiasis among school children in the area. Microscopic examination of the samples for ova showed *S. haematobium* prevalence ranging from 4.4% - 49.6% across the schools. The study showed a higher prevalence among male (40.3%) than female (19.5%) pupils, with males 3.4X more likely to be infected than females (95% CI = 1.9 - 6.0, $P < 0.001$). There was marked school absenteeism in both infected and uninfected children before treatment. School attendance did not improve for most children after treatment with praziquantel (negatives: $\chi^2 = -0.96$, $P>0.34$ and positives: $\chi^2 = -0.35$, $P>0.73$). Urinary schistosomiasis has a focal prevalence among the school-going children in Chitongo. Chemotherapy, public health education, and molluscicide are recommended to improve the long-term health of at-risk children.

306

FEASABILITY OF SCHISTOSOMIASIS MANSONI ENDEMIC EVALUATION USING EITHER SERODIGNOSTIC OF MOLECULAR DETECTION METHODS IN BURKINA FASO

Hermann Sorgho, Ollo U. Da, Jean-Bosco Ouédraogo

Institut de Recherche en Sciences de la Santé, Bobo-Dioulasso, Burkina Faso

Schistosomiasis mansoni endemic evaluation and control remain confronted to the lack of accuracy of traditional diagnostic methods. We have standardized and tested indirect haemagglutination assay (IHA) and PCR methods for the detection of *Schistosoma mansoni* infection on endemic area of Burkina. Glutaraldehyde fixed sheep red blood cells have been sensitized with either *S. mansoni* egg (SEA) or worm (SWAP) antigen preparations and later used to analyze 458 endemic sera in IHA. The same samples were submitted to *S. mansoni* DNA detection using polymerase chain reaction. All the patients were initially diagnosed using the Kato-Katz method. Our results showed high sensitivities of 93.0 % and 89.7% respectively for SEA-IHA and SWAP-IHA whereas the specificities were relatively low (41.8 % and 49.2 %). The DNA detection method showed a higher sensitivity and specificity respectively 99.0% and 95.0%.Taken together these results have proved that it was possible to adapt an efficient IHA method for endemic evaluation of schistosomiasis mansoni. Moreover they showed that sophisticated methods can be used with more satisfactory results when there are enough resources.

307

A CLOSER LOOK AT THE PROTEINS INVOLVED IN SEROTONIN SIGNALING IN *SCHISTOSOMA MANSONI* AND HOW THEY MODULATE BEHAVIOR

Nicholas Patocka, Paula Ribeiro

McGill University, Ste-anne-de-bellevue, QC, Canada

Serotonin (5:hydroxytryptamine: 5HT) has been shown to be an important modulator of neuromuscular function and metabolism in flatworms, including the bloodfluke *Schistosoma mansoni*. Exogenous application of 5HT to intact schistosomes causes contraction of the body wall musculature and a robust increase in motor activity. Lack of serum in culturing also leads to death of the parasites, suggesting a crucial role of serotonin in their development. It is unknown at the present whether the effect of exogenous serotonin is mediated by the binding to surface (tegumental) receptors leading to downstream signaling via the worm's sensory nervous system, or if it is transported by a surface carrier to act on internal receptors. Previous work showed the presence of a 5HT - specific transporter (SERT) in *S. mansoni*. The parasite SERT was shown to mediate the uptake of exogenous 5-HT in live parasites suggesting it may be

located on the surface. In addition to this transporter, we have recently identified two 5HT-like receptor sequences in schistosomes. One of these receptors was cloned and shown to respond to 5HT through the activation of cAMP when expressed in mammalian cells. Localization of the receptor *in vivo* shows it to be situated on the tegument, namely the tubercles of the adult worm, as well as weakly on day 0 schistosomula. In order to determine if either the SERT or 5HT-like receptor are mediating behavioural responses to exogenous 5HT, we developed an assay to test for mobility in cultured schistosomula. Using live imaging, we were able to quantify movement of schistosomula in the presence and absence of exogenous 5HT. Parasites are now being treated with known SERT or 5HT receptor blockers to test whether any of these drugs can inhibit the response to 5HT. The results of these pharmacological studies will be discussed. We've been successful at knocking down expression using RNAi which will be used to confirm our pharmacological results for behavioral changes, as well as to elucidate the importance of both proteins for survival and development.

308

IMPLICATIONS OF THE EFFECT OF *SCHISTOSOMA MANSONI* AND *SCHISTOSOMA HAEMATOBIUM* CO-INFECTIONS ON HUMAN MORBIDITY INDICATORS

Anouk N. Gouvras[1], Alice J. Norton[1], Curtis H. Kariuki[2], Alan Fenwick[1], Joanne P. Webster[1]

[1]Imperial College London, London, United Kingdom, [2]National Museums Kenya, Kenya, Kenya

In the developing world polyparasitism is common especially in the poverty stricken areas where hygiene and education are low and many parasites are co-endemic. Control strategies and public health measures should consider how synergistic or antagonistic interactions of co-infections may clinically affect individuals and how co-endemicity may affect the epidemiology of the parasites. *Schistosoma mansoni* and *S. haematobium*, digean trematodes infecting humans, are the most widely distributed and prevalent schistosome species in Africa. Forty countries are endemic to both species raising the likelihood of mixed schistosome species co-infections. Few studies have, however, looked at how co-infection with both *S. haematobium* and *S. mansoni* affects the development and progression of schistosomiasis. The objective of this study was to determine whether schistosome induced morbidity is different in *S. mansoni* and *S. haematobium* co-infections compared to single infections at baseline and post praziquantel treatment. Four groups of children in two neighbouring schools were entered in the study, those with *S. mansoni* only infections, those with *S. haematobium* only infections, those that were infected with both and, acting as a control group, children infected with neither species of schistosomes. *S. mansoni* related morbidity was measured by clinical examination by palpation of the liver and spleen and *S. haematobium* related morbidity by a biochemical test for urinary albumin indicative of kidney damage. Our results show no significant difference in *S. mansoni* related morbidity between *S. mansoni* only, *S. haematobium* only, co-infected and uninfected groups. However the study does indicate that some form of inter-specific interactions between *S. mansoni* and *S. haematobium* is affecting, directly or indirectly, *S. haematobium* associated morbidity. Our results to date from research in progress will be presented and discussed in terms of their implications for targeted control programmes and policy.

309

YIELD OF THREE WILD BIRD STOOL COLLECTION METHODS FOR AVIAN INFLUENZA SURVEILLANCE

Catalina Hoyos[1], Bruno M. Ghersi[2], Rodrigo Iglesias[2], Elliot Stieglitz[1], Hugo R. Razuri[3], Armando E. Gonzales[2], Andres G. Lescano[3], Joel M. Montgomery[3]

[1]Stony Brook University School of Medicine, Stony Brook, NY, United States, [2]Universidad Nacional Mayor de San Marcos, School of Veterinary Medicine, Lima, Peru, [3]U.S. Naval Medical Research Center Detachment, Lima, Peru

The threat of the emergence of an avian influenza pandemic demands continued surveillance. Studies on wild birds are limited by the low prevalence of influenza viruses and large number of samples which are needed. Environmental sampling can be a low cost alternative and several methods exist, although there is limited information about their yield. We assessed the effectiveness of three field methods for collecting fecal samples from wild birds. We collected fecal samples using plastic tarps, purposely-built platforms and direct observation-collection in two wetlands around coastal lagoons near Lima, Peru. Clean, 1.5x3m plastic tarps of varying colors were placed at dusk near large congregations of birds in both wetlands, and we collected fresh fecal samples from the tarp at dawn. Three wooden platforms were built, two on the edge and another one floating in a wetland, and the presence of stool was assessed periodically. Finally, veterinary staff followed bird colonies and collected 50 fresh fecal samples directly from the ground. Collections using the three methods were done on different dates during one month. We then compared the number of stool specimens collected with each method. Sixteen tarps were placed, half in each wetland, during five different occasions. Three tarps were stolen in Puerto Viejo, and four of the remaining 13 tarps yielded 16 samples, 2-6 samples in each tarp. The platforms were observed six times, having 0-2 samples on each platform during the first three observations and 0-6 samples in the last three visits. Direct observation produced 50 samples in 3-4 hours during the six collection dates, significantly more samples than the tarps ($p<0.001$) and platforms ($p<0.001$), and required only a few hours of work in the wetland. In conclusion, this preliminary data suggests that for waterfowl, direct observation-collection allows more efficient environmental sampling than tarps or platforms and do not require multiple/lengthy visits. In addition, it allows species identification and targeting, and adapts better to the variable displacement of wild birds. Platforms may be useful, especially for perching birds. Although environmental sampling methods is ultimately judged by viral isolation, efficient, low cost stool collection approaches to obtain many samples are needed. Further insight in diverse settings about the yield and effort needed of available sampling methods is necessary.

310

FIELD DETECTION OF EBOLA- AND MARBURG VIRUSES BY A PCR-BASED LATERAL FLOW DIPSTICK ASSAY

Roman Wölfel[1], Markus Panning[2], Gerhard Dobler[1]

[1]Bundeswehr Institute of Microbiology, Munich, Germany, [2]Bernhard-Nocht Institute for Tropical Medicine, Hamburg, Germany

Rapid identification of Ebola virus (EBOV) and Marburg virus (MBGV) is required to prevent spread of the infection in outbreak situations. We developed an one-step reverse transcription-PCR assay for the rapid detection of human pathogenic filoviruses. Detection of amplification products is based on the established immunological "lateral-flow dipstick" (LFD) technique which has been adapted to detect PCR products. Thus this approach combines the sensitivity of molecular detection with the ease and safety of handling LFDs. The amplicon created by a generic PCR is captured by a mixture of filovirus-specific probes bound on the LFD. In addition, an internal amplification control was integrated in the assay thereby allowing the detection of any sample-derived PCR inhibition in order to reduce false negative test results. The assay is able to detect

less than 20 copies of in-vitro transcribed EBOV and MBGV RNA per reaction. It was highly specific for both filovirus species, because no band was visible on the LFD when a panel of relevant other pathogens known to cause hemorrhagic fever was tested. The filovirus detection assay developed in this study is rapid, simple, specific, and sensitive for the detection of both EBOV and MBGV, and so may be an effective, field-appliable diagnostic tool for these hemorrhagic fevers. In contrast to other PCR detection platforms for the molecular diagnosis the "Filo LFD" does not require sophisticated instrumentation and needs only 10 minutes for the read-out step. It seems very suitable for diagnosis in the field or laboratories in Ebola and Marburg outbreak areas such as Central Africa.

311

VIRULENCE VARIATION AMONG ISOLATES OF WESTERN EQUINE ENCEPHALITIS VIRUS IN AN OUTBRED MOUSE MODEL

Christopher H. Logue

Centres for Disease Control and Prevention and Colorado State University, Fort Collins, CO, United States

A functional animal model is critical for detailed studies of the molecular determinants of viral pathogenicity. We analyzed infection and mortality associated with seven North American Western equine encephalitis virus (WEEV) strains in an outbred CD1 mouse model and observed significant variability in virulence patterns. The complete genome of all seven WEEV isolates was sequenced and analyzed for genetic differences. McMillan (McM) and Imperial-181 (IMP-181) were the most diverse in sequence having 66 amino acid differences of which 22 were unique to McM when compared to all 6 strains included in the investigation. Six of seven WEEV isolates induced mortality in CD1 mice following subcutaneous inoculation with 10^3 pfu of virus. The percent of mice dying (10-100%) and mean time to death or MTD (4 - 9.7 days) varied significantly among the isolates. IMP-181 virus generated no mouse mortality when inoculated at 10^3 pfu but did show a 10% mortality on day 7 following subcutaneous inoculation with 10^4 pfu. Mean peak viremia for isolates inoculated by the subcutaneous route occurred on day 1 post infection and ranged from about 3-5 $\log_{10}$ pfu/ml; peak viremia was not associated with virulence. McM was 100% lethal by aerosol by 4 days post-exposure, whereas IMP-181 showed only 10% mortality after 7 days. Mortality was accompanied by overt neurological signs and brain lesions dominated by neuronal necrosis and microvascular pahology. Isolates differed significantly in virus titer in the brain and severity of lesions as well as viral load in 16 additional organs. McM and IMP-181 were identified as high and low virulence isolates, respectively, based on morbidity, mortality, MTD, virus distribution patterns and severity of brain lesions. The work completed here will allow us to utilize infectious cDNA clones (pMcM, pIMP-181) representing high (McM) and low (IMP-181) virulence isolates to investigate molecular determinants of pathogenicity of WEEV by different routes of infection.

312

RABIES IN BATS IN TWO COMMUNITIES IN PERU AFTER AN OUTBREAK IN 2007

Gabriela Salmon-Mulanovich[1], Christian Albújar[1], Carolina Guevara[1], Alicia Vasquez[2], Alberto Laguna[1], Milagros Salazar[3], Hernán Zamalloa[1], Marcia Cáceres[4], Tadeusz Kochel[1], Carlos Contreras[4], Felix R. Jackson[5], Charles E. Rupprecht[5], Joel M. Montgomery[1]

[1]Naval Medical Research Center Detachment, Lima, Peru, [2]Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima, Peru, [3]University of Texas Medical Branch, Galveston, TX, United States, [4]Dirección de Salud, Madre de Dios, Peru, [5]Centers for Disease Control and Prevention, Atlanta, GA, United States

We conducted an ecological assessment in May 2007 after an outbreak of suspected vampire bat-related human rabies, with 23 deaths. The objective was to explore prevalence rates of rabies virus infection among vampire bats and non-vampire bats and to assess the relative distribution of bat genera from the outbreak area. Bats were netted in two locations (A and B) located near human settlements; separated by approximately 16km in the southern rainforest of Peru. Bats were euthanized for blood and tissue sample collection. Samples were tested at the CDC for rabies virus specific antibodies using a rapid fluorescent focus inhibition test (RFFIT) and brain stems were tested by direct fluorescent antibody (DFA) for evidence of active infection. Antibody prevalence and confidence intervals (CI) were calculated using the binomial exact method and genera distribution was compared with the χ^2 test. A total of 195 bats were collected; however, sufficient amount of sera for RFFIT was only available from 165 animals. Of the 165 bats, 62% were females, 85% were adults and 137 (83%) were netted in community B. Six genera, including 10 species, were represented among the sampled bats; 125 (75%) were *Carollia* sp. Seventeen bats were antibody positive to rabies virus, for an antibody prevalence of 10.3% (95% CI: 6.1; 16.0). Antibody positive response was similar (p=0.859) among vampire bats (1/7, 14%), *Carollia* sp (12/125, 10%) and other non-vampire genera (*Uroderma*, *Sturnira*, *Platyrhinus,* and *Artibeus*) (4/33, 12%). No bats were positive by DFA. Bats from the genus *Carollia* sp. were collected more frequently from natural, non-disturbed habitats (eg. creeks, caves) while other non-vampire and vampire bats were found in more visibly disturbed habitats (eg. plantations, cattle farms) (p<0.001). None of the bats had active infection, but both vampire and non-vampire bats had antibodies to rabies virus, suggesting cross-species transmission. This has important implications in the public health approach to prevent sylvatic rabies in the rainforest from potential exposure to non-vampire bats, which are more abundant.

313

CORTICOSTEROIDS MODULATE SEOUL VIRUS INFECTION, REGULATORY T CELL RESPONSES, AND MMP-9 EXPRESSION IN MALE, BUT NOT FEMALE, NORWAY RATS

Judith D. Easterbrook, **Sabra L. Klein**

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

Human hantaviral disease is mediated by excessive proinflammatory and CD8+ T cell responses, which can be alleviated by administration of corticosteroids. In contrast to humans, male rats that are infected with their species-specific hantavirus, Seoul virus (SEOV), have reduced proinflammatory and elevated regulatory T cell responses in tissues where virus persists. To determine the effects of glucocorticoids on SEOV persistence and immune responses during infection, male and female Norway rats received sham surgeries (sham) or were adrenalectomized (ADX0), in some of which corticosterone was replaced at low (ADX10) or high (ADX80) doses. Rats were inoculated with SEOV and serum corticosterone, SEOV RNA, gene expression, and protein production were measured at different timepoints post-inoculation (p.i.). We observed that SEOV infection suppressed corticosterone in sham males to concentrations seen in ADX0 males. Furthermore, males with low corticosterone had more SEOV RNA in the lungs than either females or males with high corticosterone concentrations during peak infection. Although high concentrations of corticosterone suppressed the expression of innate antiviral and proinflammatory mediators to a greater extent in females than males, these immunomodulatory effects did not correlate with SEOV load. Males with low corticosterone concentrations and high viral load had elevated regulatory T cell responses and expression of matrix metalloprotease (*Mmp*)*9*. MMP-9 is a glycogenase that disrupts cellular matrices and may facilitate extravasation of SEOV-infected cells from circulation into lung tissue. Suppression of glucocorticoids may, thus, contribute to more efficient dissemination of SEOV in male than female rats.

314

DETECTION OF VIRAL RNA FROM PARAFFIN-EMBEDDED TISSUES AFTER PROLONGED FORMALIN FIXATION

Randal J. Schoepp[1], Michelle D. McKinney[2], Steven J. Moon[1], David A. Kulesh[1], Thomas Larsen[1]

[1]U.S. Army Medical Research Institute for Infectious Diseases, Frederick, MD, United States, [2]Geo-Centers, Inc., Frederick, MD, United States

Isolating amplifiable RNA from formalin-fixed, paraffin-embedded (FFPE) tissues is more difficult than isolating DNA because of the potential for RNA degradation by RNases, chemical modification of the RNA by addition of methylol groups (-CH_2OH), and cross-linking of nucleic acids and proteins during the fixation process. To improve procedures for extracting RNA from these extensively FFPE tissues and detect the RNA with the more sensitive fluorogenic probe-based PCR assays. Through modifications of a commercially available kit, we were able to extract amplifiable RNA from FFPE tissues and detect West Nile virus (WNV), Marburg virus (MARV), and Ebola virus (EBOV)-infected tissues using fluorogenic 5' nuclease (TaqMan®) reverse transcriptase (RT)-PCR assays. Formalin fixation results in an approximately 2 $\log_{10}$ reduction in detection limit when compared to fresh tissues. Increasing proteinase K digestion (24 hr) improved extraction of amplifiable RNA from FFPE tissues. The TaqMan® results were comparable to more traditional detection results such as virus isolation. This improved extraction procedure for obtaining amplifiable RNA combined with the more sensitive and specific fluorogenic probe-based RT-PCR assays will now permit retrospective and prospective studies on FFPE tissues infected with BSL-3 and -4 pathogens.

315

FULL LENGTH SEQUENCING AND GENETIC CHARACTERIZATION OF BREU BRANCO VIRUS (BE AR 494347) AND STRAINS BE AR 494475 AND BE AR 486204 ISOLATED FROM ANOPHELES MOSQUITOES

Conceição M. Vieira[1], Márcio R. Nunes[2], Eliana V. da Silva[2], Valéria L. Carvalho[2], Joaquim P. Nunes Neto[2], Helena B. Vasconcelos[2], Ana C. Cruz[2], Samir M. Casseb[2], **Pedro F. Vasconcelos**[2]

[1]Universidade Federal Rural da Amazônia, Belém, Brazil, [2]Instituto Evandro Chagas, Belém, Brazil

The prototype strain of the Breu Branco virus (BE AR 492347) was isolated from *Anopheles (Nys.) triannulatus* mosquitoes captured in Tucuruí, Pará state in 1988. No cross-reactivity by complement fixation tests was observed between Breu Branco virus and other known Brazilian arboviruses. Results of electronic microscopy and physicochemical test (DCA) suggested Breu Branco virus as a possible member of the genus *Orbivirus*. In order to elucidate its taxonomic status, a comprehensive study on genetic characterization, including full-length genome sequencing, determination of the genetic traits and phylogenetic analysis, was conducted for Breu Branco virus and related strains (BE AR 494475 and BE AR 486204), also isolated from *Anopheles* mosquitoes in the same area. As results, Breu Branco virus showed similar genome organization (10 RNA segments) in comparison to members of the genus *Orbivirus*, family *Reoviridae*. Genetically, Breu Branco virus was indistinguishable from the strains BE AR 494475 and BE AR 486204 and related to members of the genus *Orbivirus.* The ten RNA segments were sequenced and the phylogenetic analysis suggested that Breu Branco virus and related strains constitute a different orbivirus species. Finally, Breu Branco virus represents the first Brazilian orbivirus completely sequenced and the fifth in the world.

316

SEROPREVALENCE RATES OF MAYARO VIRUS IN URBAN AND RURAL AREAS OF MAYNAS PROVINCE, PERU

Kanya C. Long[1], Amy C. Morrison[2], Brett M. Forshey[3], Alfredo Huaman[3], Claudio Rocha[3], Rebeca Carrion[3], Cristian Carey[4], Joel M. Montgomery[5], Robert B. Tesh[1], Tad Kochel[3]

[1]University of Texas Medical Branch, Galveston, TX, United States, [2]University of California, Davis, Davis, CA, United States, [3]Naval Medical Research Center Detachment, Lima, Peru, [4] Dirección Ejecutiva de Epidemiología de Salud de Loreto, Iquitos, Peru, [5]US Centers for Disease Control, Atlanta, GA, United States

Mayaro virus (MAYV) is a mosquito-borne alphavirus found in tropical forests of South and Central America that can cause an acute febrile illness with severe arthralgia. In Iquitos, Peru, transmission of MAYV has been documented regularly since 1993 through a clinic-based febrile surveillance program, with an average of four acute MAYV cases per year. To begin to understand transmission patterns of MAYV, we conducted a seroprevalence study for antibodies against MAYV among residents of Maynas Province in northeastern Peru. Blood samples, demographic information and behavioral data were collected from 1198 residents of urban Iquitos during 2006 and from 3000 residents of Iquitos and rural communities surrounding the city during 2008. Preliminary screening by IgG ELISA of serum samples collected during 2006 indicates a MAYV seroprevalence rate of 16.2% among residents of urban areas of Iquitos. MAYV infection rates were stratified by age and odds ratios determined by multivariate logistic regression. Antibody prevalence increased with age (>18y years of age, OR=1.81, 95% CI of 1.32-2.46), and an elevated risk for MAYV infection was associated with living in neighborhoods adjacent to the Nanay and Itaya Rivers (OR=1.46), specific occupations (OR=3.10) and recent travel (OR=1.49). Due to cross-reactivity among co-circulating alphaviruses, these data require confirmation by plaque reduction test. Nevertheless, our preliminary results suggest high levels of MAYV transmission within or around Iquitos. Based on the ecology of previously identified MAYV vectors and hosts, we expect that seroprevalence rates will be higher across all categories in rural populations included in the study.

317

SINDBIS ALPHAVIRUS INFECTION: CLINICAL FEATURES, DIAGNOSIS AND EPIDEMIOLOGY

Satu Kurkela[1], Tapani Helve[2], Osmo Rätti[3], Tytti Manni[1], Eili Huhtamo[1], Nathalie Yumari Uzcátegui[1], Johanna Myllynen[4], Juha Laakkonen[5], Juha Pekka Nuorti[6], Antti Vaheri[1], Olli Vapalahti[1]

[1]Haartman Institute, University of Helsinki, Helsinki, Finland, [2]Helsinki University Central Hospital, Helsinki, Finland, [3]Arctic Centre, University of Lapland, Rovaniemi, Finland, [4]Helsinki University Central Hospital Laboratory, Helsinki, Finland, [5]Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland, [6]National Public Health Institute, Helsinki, Finland

Sindbis virus (SINV) (genus *Alphavirus*) or antibodies to SINV are widely found in insects and vertebrates in Eurasia, Africa, and Oceania, but clinical infection, characterized by rash-arthritis, only occurs in few geographically restricted areas. Since 1974, for unknown reason, the disease has occurred as large outbreaks every seven years in Finland, Northern Europe. This study is based on the material collected during and following the 2002 outbreak in Finland. We developed and characterized SINV IgM and IgG EIAs, based on purified SINV, to be used in serodiagnostics. We studied 86 patients with SINV infection. The acute disease consisted of arthritis, rash, fatigue, mild fever, headache, and muscle pain. Physical examination of 49 of these patients 3 years after infection revealed persistent joint manifestations: 2 patients had arthritis, and a total of 24.5% had objective and/or subjective joint manifestations attributable to the infection. A positive IgM antibody response persisted in 3/49 of the patients; both two patients with arthritis were in this group.

RNA detection or virus isolation was successful in skin and/or whole blood specimens of 8 acute-phase patients. These Finnish SINV strains represent the first human SINV isolates from Europe, and seem to have a common ancestor with strains from South-Africa, where clinical cases have been sporadic. The redistribution of SINV likely occurs in a longitudinal direction, possibly with migratory birds. We serologically studied resident grouse (N=621), whose population declines have previously coincided with human SINV outbreaks, and migratory birds (N=836). SINV HI antibodies were found for the first time in birds during their spring migration to Northern Europe; from three individuals: red-backed shrike, robin, and song thrush. SINV seroprevalence in grouse declined from 27.4% in 2003 (1 year after outbreak) to 1.4% in 2004; grouse might contribute to the human epidemiology of SINV. The seroprevalence of SINV in Finnish population was shown to be 5.2% (1999-2003).

318

NORTH AND SOUTH AMERICAN EASTERN EQUINE ENCEPHALITIS VIRUS INFECTION OF HISPID COTTON RATS

Nicole C. Arrigo, Patrick C. Newman, A. Paige Adams, Douglas M. Watts, Scott C. Weaver

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

Eastern Equine Encephalitis Virus (EEEV) is an arbovirus in the genus *Alphavirus*, family *Togaviridae*. There are two major EEEV subtypes; one in Central and South America (SA EEEV) that is associated with equine but not human disease, and one in North America (NA EEEV) that is associated with severe human and equine neurological disease. Despite the epidemiologic dichotomy between NA and SA EEEV, little is known about their ecological differences. It remains unclear whether SA EEEV utilizes avian amplifying hosts in its enzootic transmission, like its genetic sister NA EEEV, or ground-dwelling mammals, like its genetic and sympatric cousin, VEEV. In order to clarify the role of ground-dwelling mammals in the transmission of SA EEEV, the infection dynamics of hispid cotton rats, *Sigmodon hispidus*, with NA and SA EEEV were compared. Cotton rats collected in Galveston, TX, were divided into three experimental cohorts based on age. Within each cohort, two or three infection groups were established and each subcutaneously inoculated with one of three EEEV viruses: FL93-939 (NA EEEV), C-49 (SA EEEV), or PE70 77U1104 (SAEEEV). Animals were weighed and bled daily for 7 days or until moribund and the blood tested by plaque assays to determine intensity and duration of viremia. Antibody profiles and seroconversion were also established via PRNT. Upon death or sacrifice, tissue were taken to determine viral load and processed for histopathology and immunohistochemistry. Despite overall variation with age, viremia levels were comparable between NA and SA EEEV and all age groups developed viremia of sufficient intensity and duration to potentially infect EEEV mosquito vectors. NA EEEV resulted in high mortality rates in adult cotton rats, while SA EEEV did not result in death or observable illness. These results suggest that *Sigmodon hispidus*, and potentially other ground-dwelling mammals, could potentially serve as amplifying hosts in the transmission of SA EEEV.

319

TRANSMISSION OF NIPAH BY DATE PALM SAP, BANGLADESH 2008

Muhammad Aziz Rahman[1], M. Jahangir Hossain[2], Sharmin Sultana[3], Shahed Sazzad[2], Nusrat Homaira[1], Sayma Afroze[3], Mahmudur Rahman[3], Emily Gurley[2], Stephen P. Luby[4]

[1]International Center for Diarrhoeal Disease Research, Bangladesh and IEDCR (Institute of Epidemiology, Disease Control and Research), Dhaka, Bangladesh, [2]International Center for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [3]IEDCR (Institute of Epidemiology, Disease Control and Research), Dhaka, Bangladesh, [4]International Center for Diarrhoeal Disease Research, Bangladesh and Centers for Disease Control and Prevention-Atlanta, Georgia, USA, Dhaka, Bangladesh

Drinking raw date palm sap was associated with Nipah illness in 2005 in Bangladesh. We investigated a cluster of patients with encephalitis, identified on 27 February 2008 through our ongoing Nipah surveillance, in Manikgonj and Rajbari districts of Bangladesh to determine the etiology and identify the risk factors for that illness. We conducted a case-control study. We classified confirmed Nipah cases by the presence of immunoglobulin M antibodies to Nipah virus in serum and probable cases as persons who developed fever with new onset of seizures or altered mental status from 6 February 2008 through 10 March 2008, but who died before blood was collected. For each case we selected four unmatched controls from nearby households and collected information about their exposures. Proxy respondents answered for deceased cases. We identified a total of ten cases (4 confirmed and 6 probable). Nine of them died (case fatality ratio 90%). The median age of cases was 10 years; 8 were males. The outbreak occurred at the same time in two adjoining districts, about 50 kilometers apart and separated by a river. Cases were more likely to have consumed date palm sap within one month prior to their illness than controls (100% among case-patients vs. 25% among controls, OR undefined, $p<0.001$). All the cases in the two clusters drank fresh date palm sap and each cluster consumed the sap from the same collection pot a week apart. Median incubation period was 6 days (2-12 days). Most of the cases consumed date palm sap just for once (60% vs. 8%, $p<0.001$) and less than one glass per day within one month prior to the onset of their illness (60% vs. 5%, $p<0.001$). All the cases consumed the sap before 9 am (100% vs. 23%, $p<0.001$). In conclusion, our investigation suggests that those nine people died from Nipah infection after drinking date palm sap in two separate locations within a five week period. This is the second Nipah outbreak in Bangladesh where date palm sap was implicated. Efforts to prevent bat access to date palm sap collection could reduce Nipah transmission to humans.

320

EVALUATION OF RISK FOR AVIAN INFLUENZA INTRODUCTION USING GIS IN WETLANDS IN PERU

Hugo R. Razuri[1], Bruno M. Ghersi[1], Veronica Landa[2], Gabriela Salmon-Mulanovich[1], Jorge Pastor[2], Raul Zegarra[2], David L. Blazes[1], Joel Montgomery[1], Andres G. Lescano[1]

[1]Naval Medical Research Center Detachment, Lima, Peru, [2]National Animal and Plant Health Service, Ministry of Agriculture, Lima, Peru

Wetlands within transcontinental migratory paths can play an important role the introduction of avian influenza (AI) due to contact between migratory birds, local wild birds and backyard flocks, and the proximity of large human populations. We assessed this potential risk in Peru using geographic information systems (GIS). From January to June 2008 we visited the 10 largest coastal wetlands located within 300 km from Lima, Peru. We used 12-channel recreational GPS units to record the wetland and water mirror perimeters, and the location of commercial poultry and porcine farms and human populations within a 5 km radius of each wetland. Trained personnel recorded and photographed all wild bird species found in each wetland. The Ministry of Agriculture provided data about the size and biosafety level of each commercial poultry farm,

and all these Information was integrated in a geographic information system using ArcView 9.2 and Google Earth. Individual, detailed wetland maps were prepared, calculating the minimal distance from the wetland to human settlements, swine and poultry farms. A risk score for each wetland was constructed based on presence, size and proximity of human population poultry and swine farms.The water surface of the wetlands ranged from 0.1 -2.6 square kilometers. We observed between 20-30 wild bird species in each wetland, including at least 15 known transcontinental migratory birds. Similar species were observed across all wetlands. We found risk scenarios that could allow transmission of avian influenza in eight of the ten wetlands. Commercial poultry farms were located <500m from two wetlands and human settlements were observed within 100 to 500m from eight of the ten wetlands sampled. Five wetlands were located within a 5000m radius of a human settlement with a population of >1000 inhabitants. Backyard ducks and a porcine farm were located on the interior of one wetland and within 500m of another, respectively. Commercial poultry farming and human populations were found in close proximity to nearly all main coastal wetlands in Peru. This confirms wetlands as potential risk sites for AI introduction in Peru. Human populations and backyard and commercial poultry farming operations in close proximity to wetlands should be prioritized for AI surveillance and intervention strategies.

321

DICISTRONIC EXPRESSION OF MULTIPLE FLUORESCENT PROTEINS FROM A DOUBLE SUBGENOMIC ALPHAVIRUS

Michael R. Wiley[1], Lisa O. Roberts[2], Zach N. Adelman[1], Kevin M. Myles[1]

[1]Virginia Tech, Blacksburg, VA, United States, [2]School of Biomedical and Life Sciences, University of Surrey, Guildford, United Kingdom

Members of the genus Alphavirus are typically maintained in transmission cycles involving an arthropod vector and susceptible vertebrate host. The genome of the type alphavirus, Sindbis (SINV), is a positive-sense, non-segmented, single-stranded RNA 11,703 nucleotides in length. The 5′ two-thirds of the genome encode the nonstructural proteins, while the structural proteins are translated from a subgenomic mRNA (26S) that is collinear with the 3′ one-third of the genome. Alphavirus expression systems can be generated by duplicating the subgenomic promoter in the viral genome. Exogenous proteins inserted downstream of the duplicated viral promoter can be expressed at high levels in an extensive range of species and tissues. For this reason, double subgenomic (ds) alphaviruses have become an important tool in the study of virus-vector interactions. However, a major limitation of double subgenomic alphaviruses is that only one exogenous gene can be expressed from the duplicated promoter. Rhopalosiphum padi virus (RhPV; family Dicistroviridae) uses internal ribosome entry sites (IRES) to initiate cap-independent translation of its nonstructural and structural polyproteins. To determine if more than one exogenous protein could be expressed from the dsSINV, TE 3′2J, the RhPV 5′ UTR IRES was inserted between a green fluorescent protein (GFP) and a red fluorescent protein (DsRED) downstream of the second subgenomic promoter. Expression of both GFP and DsRED were observed in cultured mosquito and vertebrate cell lines, as well as adult *Aedes aegypti* infected with TE 3′2J/GFP-IRES-dsRED virus. This work demonstrates that the RhPV IRES can be used to express multiple proteins from a single subgenomic mRNA. Alphavirus expression systems incorporating the RhPV IRES will now be a valuable tool in the study of virus-vector interactions.

322

MALARIA POTENTIATES EXPERIMENTAL MYCOBACTERIAL INFECTION *IN VITRO* AND *IN VIVO*

Michael Hawkes, Xiaoming Li, Maryanne Crockett, Angelina Diassiti, W. Conrad Liles, Jun Liu, Kevin Kain

University of Toronto, Toronto, ON, Canada

Tuberculosis (TB) and malaria are among the most important infectious causes of mortality worldwide, accounting for an estimated 1.5 million and 1 million deaths every year, respectively. Tropical and developing countries carry the heaviest burden of both of these infectious diseases, and malaria-TB co-infection is likely to occur in individuals living in these zones of intense transmission; however, little is known about the interaction of mycobacteria and malaria within the mammalian host. Using *in vitro* and *in vivo* experimental models, we show that infection with *Plasmodium* spp. exacerbates infection with a range of mycobacterial species. Co-incubation of *Mycobacterium marinum* and *P. falciparum* with the human macrophage cell line RAW 264.7 resulted in higher bacterial counts than *M. marinum* alone. Likewise, primary murine macrophages co-incubated with *M. tuberculosis* and *P. falciparum* yielded higher bacterial counts than *M. tuberculosis* alone. *In vivo*, co-infection with *M. bovis* BCG and the murine malaria parasite *P. chabaudi chabaudi* AS (PCCAS) resulted in higher BCG loads in the organs of infected mice than infection with BCG alone, as well as higher levels of the pro-inflammatory cytokines tumor necrosis factor and interferon-γ. Histopathologic examination of the infected organs revealed heavy deposition of malaria pigment within the granulomata of co-infected mice, suggesting that macrophages internalizing parasitized erythrocytes co-localize to the site of mycobacterial control. In mice latently infected with BCG, challenge with PCCAS resulted in transient re-activation of BCG infection, with disruption of granuloma structure. Collectively, these results identify a biological interaction of malaria and mycobacteria at the level of the macrophage, the phagocyte common to both pathogens. These findings may have significant implications for human populations, where the interaction of these globally important pathogens may potentiate TB infection and transmission.

323

IMPACT OF HIV-1 ON HUMORAL IMMUNITY TO *PLASMODIUM FALCIPARUM* MALARIA IN NON-PREGNANT ADULTS WITH UNCOMPLICATED MALARIA IN ZAMBIA

Erica Van Eijk[1], **Jean-Pierre Van geertruyden**[2], Francisca Yosaatmadja[3], Webster Kasongo[4], Modest Mulenga[4], Umberto D'Alessandro[2], Stephen Rogerson[3]

[1]Vrije Universiteit Amsterdam, Amsterdam, Netherlands, [2]Prince Leopold Instituut voor tropische geneeskunde, Antwerpen, Belgium, [3]Melbourne University, Melbourne, Australia, [4]Tropical Disease Research Centre, Ndola, Zambia

HIV-1 infected individuals with low CD4 count have a higher prevalence of malaria parasitaemia, uncomplicated or severe malaria, and are more at risk of treatment failure. HIV-1 infection appears to affect malaria humoral immunity during pregnancy, but data are lacking for non-pregnant adults. We assessed if HIV-1 infection affects malaria humoral immunity in adults with clinical malaria and if humoral immune suppression is a risk factor for treatment failure. In 224 HIVneg and 115 HIVpos samples, ELISAs were performed to determine IgG levels against blood stage merozoïte antigens AMA1, MSP2 serotype A and B. Antibodies to the Variant Surface Antigens (VSA) of 3 different parasite lines E8B, A4 and HCD6 were measured by flow cytometry. HIV-1 infected individuals had lower AMA-1 IgG (p=0.02) than non HIV-1 infected, but did not differ on both MSP2 serotypes (p=0.68 and p=0.14). HIV-1 infected had a low IgG to all merozoite antigens combined (p=0.02). Within HIV-1 infected, CD4+count at day 0 was positively associated with AMA-1 antibody (p= 0.003) but not with MSP 2 serotypes (p=0.86 and p=0.38). Parasite load was negatively associated with antibodies to MSP-2 serotypeA (p=0.02)

and MSP serotype B (p= 0.08). Low IgG levels to all merozoite antigens combined was associated with higher parasite burden (p=0.01) and lower CD4 count (p=0.03). HIV-1 infected and uninfected individuals had similar levels of antibodies for all three VSA (E8B p=0.22 A4 p=0.39 HCD6 p=0.89). Within the HIV-infected, parasite load was negatively associated with antibody to E8B, A4, and HCD6 (p=0.02,p=0.02 p=0.04). VSA E8B and A4 antibodies were inversely related to CD4 count measured 45 days after malaria treatment (both P=0.02). Overall, high level of antibodies to the VSA's of E8B, A4, and HCD6 were associated with successful treatment (p=0.02, p=0.02, p=0.04). In conclusion, HIV-1 infected malaria patients had a lower level of specific malaria humoral immunity than non HIV-1 infected. Furthermore, antibodies against AMA-1, a malaria vaccine candidate, were lower in HIV-1 infected malaria patients, and associated with CD4 count. Antibodies to VSAs seem to be directly involved in controlling blood stage parasites and might compensate for impaired cellular immunity.

324

CHILD MALNUTRITION AT THE ONSET OF MALARIA TRANSMISSION: IMPACT ON SUBSEQUENT MALARIA MORBIDITY AND ANTI-*PLASMODIUM FALCIPARUM* ANTIBODY RESPONSE

Florie Fillol[1], Jean Birame Sarr[2], Franck Remoue[3], Denis Boulanger[1], Badara Cisse[4], Cheikh Sokhna[3], Geoffrey Targett[5], Jean-François Trape[3], François Simondon[1], Brian Greenwood[5], Kirsten Simondon[1]

[1]Institut de Recherche pour le Développement (IRD), Montpellier, France, [2]Association Espoir Pour la Santé (EPLS), Saint-Louis, Senegal, [3]Institut de Recherche pour le Développement (IRD), Dakar, Senegal, [4]Université Cheikh Anta Diop (UCAD), Laboratory of Parasitology, Dakar, Senegal, [5]London School of Hygiene and Tropical Medicine, London, United Kingdom

In sub-Saharan Africa, malaria and malnutrition remain major causes of morbidity and mortality of preschool children. Malnutrition is widely recognized to compromise immune function resulting in higher risk of infection. However, few studies have investigated the relationship between malaria, malnutrition and specific immunity. To explore the influence of nutritional status at baseline on the susceptibility to malaria morbidity and to anti-*Plasmodium falciparum* immune response, a cohort of 874 rural Senegalese preschool children was followed during one season of malaria transmission (July-December). Nutritional status was assessed in July and malnutrition was defined as stunting (height-for-age < -2 z-scores) and wasting (weight-for-height < -2 z-scores). In mixed models, wasting decreased the risk of experiencing at least one subsequent clinical malaria attack (OR=0.33 (95% CI: 0.13, 0.81)) whereas no significant association was observed for stunting. Each malnourished child was pair-matched to a control based on age, sex and residence; and IgG antibody response to *P. falciparum* whole extracts (schizont antigens) was assessed by ELISA. Stunted children had significantly lower IgG response compared to their counterparts (n=150, P=0.03), whereas no significant difference was observed for wasted children (n=27, P=0.4). Further analyses, involving assessment of IgG isotypes, are required to evaluate the relationship between malnutrition, anti-malaria antibody response and susceptibility to malaria. Nevertheless, these results indicate that child malnutrition at the onset of malaria transmission is associated with the risk of subsequent malaria attack and might also down-regulate anti-malaria immune response. Moreover, these effects appear to be closely dependent on the type of malnutrition (wasting versus stunting) affecting children in malaria endemic areas.

325

A LONGITUDINAL STUDY OF THE ACQUISITION AND MAINTENANCE OF *PLASMODIUM FALCIPARUM*-SPECIFIC MEMORY B CELLS

Greta Weiss[1], Boubacar Traore[2], Safiatou Doumbo[2], Didier Doumtabe[2], Younoussou Kone[2], Marko Mircetic[1], Aissata Ongoiba[2], Kassoum Kayentao[2], Ogobara K. Doumbo[2], Susan K. Pierce[1], Peter D. Crompton[1]

[1]National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Immunogenetics, Bethesda, MD, United States, [2]Malaria Research and Training Center, Faculty of Medicine, Pharmacy and Dentistry, University of Bamako, Bamako, Mali

Seroepidemiological studies in malaria endemic areas have shown that despite persistent exposure to *Plasmodium falciparum*, certain anti-*P. falciparum* antibodies are inconsistently present, relatively short-lived, or fail to boost or isotype-switch after acute infection--phenomena that may contribute to the slow acquisition and ostensibly rapid loss of clinical immunity to malaria. The cellular basis of these observations, and the role *P. falciparum* may play in modulating this response is poorly understood. Recent advances permit the phenotypic and functional characterization of memory B cells and plasma cells that are responsible for the maintenance of long-term humoral immunity. With the objective of bringing these advances to bear in a field study of malaria, we enrolled 225 individuals (aged 2-25 years) in a longitudinal study in the rural village of Kambila, Mali just prior to the 6 month malaria transmission season. During the 1 year study we recorded the malaria incidence, and by multivariate analysis determined that increasing age, sickle-cell trait, and asymptomatic parasitemia at enrollment were factors associated with decreased malaria risk. Having characterized this cohort clinically and epidemiologically, we are performing immunological analyses of peripheral blood mononuclear cells and plasma collected before, during, and after the malaria season, 14 days after the first malaria episode, and at the end of the 6 month dry season, a period of little or no malaria transmission. Assays include ELISPOT for the detection of AMA1-, MSP1-, tetanus-specific, and total IgG memory B cells; ELISA for the measurement of corresponding antibodies; and flow cytometry for the enumeration of naïve B cells, memory B cells, and plasma cells. By coupling well-characterized longitudinal clinical and epidemiological data with recent advances in basic B cell immunology, this study is providing valuable insight into the cellular basis of the humoral immune response to *P. falciparum*, information that may facilitate what has proved to be a formidable challenge, the development of an effective malaria vaccine.

326

IMMUNITY TO *PLASMODIUM FALCIPARUM* MEASURED BY GROWTH INHIBITION ASSAY DECREASES WITH AGE AND IS ASSOCIATED WITH DELAYED TIME TO BLOOD STAGE INFECTION IN NATURALLY EXPOSED PERSONS

Arlene E. Dent[1], Elke Bergmann-Leitner[2], Danny Wilson[3], Daniel Tisch[1], Rhonda Kimmel[4], John Vulule[5], Peter Sumba[5], James Beeson[3], Evelina Angov[2], Ann Moormann[1], James Kazura[1]

[1]Case Western Reserved University, Cleveland, OH, United States, [2]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [3]Walter and Eliza Hall Institute, Parkville, Australia, [4]Case Western Reserve University, Cleveland, OH, United States, [5]Kenya Medical Research Institute, Kisumu, Kenya

Antibodies that impair invasion and intra-erythrocytic growth of *Plasmodium falciparum* (Pf) are likely one of several components of acquired immune protection from malaria infection and disease. Correlating serologically measured antibodies directed against recombinant proteins with biologically relevant outcomes has been difficult and inconsistent. Growth inhibition assays (GIA) offer a convenient method to quantify the *functional* activity of antibodies directed against blood stage malaria. We compared various GIA methodologies and

examined GIA results as a surrogate of protection from blood stage parasitemia in a population where age-related acquired immunity to malaria develops. A treatment-time-to-infection study was conducted over a 12-week period in 197 residents of a high malaria transmission area of western Kenyan. Plasma collected before anti-malarial drug cure of blood stage parasitemia was tested in GIA performed in three different laboratories with their preferred methodologies. Median GIA levels varied with Pf line (D10, 8.8%; 3D7, 34.9%; FVO, 51.4% inhibition). The magnitude of growth inhibition decreased with age in all Pf lines tested with the highest median levels among children < 4 years compared to adults (e.g. 3D7, 45.4% vs. 30.0% respectively, p=0.0003). Time-to-infection measured by weekly blood smears was significantly associated with level of GIA controlling for age. Upper quartile inhibition activity was associated with less risk of infection compared to individuals with lower levels (e.g. 3D7, hazard ratio=1.535, 95% CI=1.012-2.329; p=0.0438). Culture conditions, methods of sample processing and *in vitro* endpoint measures of parasite growth had little effect on inhibition levels. Growth inhibition decreases by age but may be a useful surrogate of protection against blood stage Pf in naturally immune populations.

327

COMPARISON OF SEROLOGICAL PROFILES AND ANTIBODY AVIDITIES TO EIGHT MAJOR CANDIDATE VACCINE ANTIGENS IN THAI AND CAMEROON ADULTS

Alexander K. Kayatani[1], Mark M. Fukuda[2], Rose G. Leke[3], Diane W. Taylor[1]

[1]University of Hawaii, Honolulu, HI, United States, [2]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [3]University of Yaounde I, Yaounde, Cameroon

The frequency of *Plasmodium falciparum* transmission is thought to influence the acquisition of immunity to malaria. In Cameroon where *P. falciparum* is hyper-endemic, adults have developed immunity to severe malaria. However, in the North-Western border regions of Thailand, where transmission is unstable and seasonal, adults lack frequent exposure and remain at relatively higher risk for severe disease and death. This study compared the humoral immune responses of Cameroon (~8000 infections) and Thai (~1-2 infections) adults to *P. falciparum* antigens currently being developed for vaccine use (AMA-1, EBA-175, MSP-1 42, MSP-2, MSP-3, RESA, CSP and LSA-1). Using the Luminex xMap multiplexing technology, the levels of IgM, IgG, IgG_1, IgG_2, IgG_3, IgG_4 and IgE, as well as antibody avidities of the 8 antigens were determined. The proportion of antibody-positive Thais (n=58) and Cameroons (n=60) studied were similar for each of the antigens. Although the Cameroonians had many more infections than the Thais, they had similar IgM levels and, surprisingly, lower IgG levels against AMA-1 ($p < 0.001$), EBA-175 ($p < 0.001$), MSP-1 ($p < 0.001$), MSP-3 ($p < 0.001$), RESA ($p < 0.001$), CSP ($p < 0.01$) and LSA-1 ($p < 0.01$) when compared to the Thai samples. The isotype and subclass distribution varied among antigens but not between geographic regions. Finally, comparable antibody avidities were found in Cameroonians and Thais with the exception of AMA-1, which was higher in the Cameroonians ($p < 0.001$). In conclusion, adults studied from the hyper-endemic region of Cameroon and the holo-endemic region of Thailand had similar serological profiles with respect to seropositivity, amounts, isotype and subclass distribution and avidity to the 8 malaria antigens studied. Therefore, these findings suggest that multiple infections may not result in higher levels of antibodies or increased antibody avidity.

328

DIFFERENCES IN TRANSMISSION INTENSITIES OF FALCIPARUM MALARIA AFFECT THE FREQUENCY OF HUMAN COMPLEMENT RECEPTOR 1 (CR1) POLYMORPHISMS IN NORTH-EASTERN TANZANIA

Helle H. Hansson[1], Lasse S. Vestergaard[2], Martha M. Lemnge[3], Bruno P. Mmbando[3], Anders Enevold[1], Mette L. Schousboe[1], John P. Lusingu[3], Thor G. Theander[1], Ib C. Bygbjerg[4], Michael Alifrangis[1]

[1]Center for Medical Parasitology, University of Copenhagen and Rigshospitalet, Copenhagen, Denmark, [2]Department of Infectious Diseases, Rigshospitalet, and Institute of International Health, Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark, [3]National Institute for Medical Research, Tanga, United Republic of Tanzania, [4]Institute for International Health, Immunology and Microbiology, University of Copenhagen and Rigshospitalet, Copenhagen, Denmark

It is still unknown why some children become severely ill from malaria while others are only mildly affected or remain asymptomatic. The complement receptor one (CR1) expressed on human erythrocytes is involved in rosetting and impaired or altered function of this receptor might confer some protection against cerebral malaria. The Knops blood group system, which include the Swain-Langley (Sl) and McCoy (McC) alleles are known to lie within the CR1 protein repeats. It has been hypothesized that two altered alleles observed in Africa, Sl2 and McC^b, may be responsible for the advantageous effect. If polymorphisms in CR1 confer protection against cerebral malaria it would be expected that the frequency of these polymorphisms can be associated with malaria transmission as seen for other human polymorphisms such as the sickle cell trait and α-thalassaemia. The objective of this study was to determine and compare the genetic frequency of the CR1 polymorphisms of the Sl and McC alleles in samples from populations living in two villages with high and low malaria transmission intensities in Korogwe District, Tanzania. Single nucleotide polymorphisms (SNPs) in the CR1 were detected by PCR followed by a sequence specific oligonucleotide probe (SSOP) - ELISA method. High frequencies of the Sl2 allele were seen in both villages compared to Caucasian populations ($P \leq 0.001$). Furthermore, significantly higher frequencies of the Sl2 allele was found in the village with high transmission compared to village with low transmission ($P=0.003$), though, only significantly in individuals above five years of age ($P \leq 0.001$). For the McC^b allele, no differences between the villages were found. The results indicate that the high frequency of the Sl2 allele in Tanzania may have been selected for by malaria. This supports previous findings suggesting a protective role of this polymorphism. CR1 is an important rosetting receptor and the Sl2 allele can possibly diminish disease severity. Further studies of CR1 in the pathogenesis of cerebral malaria, appear to be justified.

329

ZOONOTIC TRANSMISSION OF *SCHISTOSOMA JAPONICUM* IN CHINA AND THE PHILIPPINES

James W. Rudge[1], Da-bing Lu[1], Maria-Gloria Basanez[1], Tianping Wang[2], Helene Carabin[3], Ernesto Balolong Jr[4], Stephen T. McGarvey[5], Joanne P. Webster[1]

[1]Imperial College London, London, United Kingdom, [2]Anhui Institute of Parasitic Diseases, Wuhu, China, [3]University of Oklahoma, Oklahoma City, OK, United States, [4]Research Institute for Tropical Medicine, Muntinlupa, Philippines, [5]Brown University, Providence, RI, United States

Control of *Schistosoma japonicum* is greatly hindered by the generalist nature of this parasite, with over 40 species of wild and domesticated animals suspected as reservoir hosts. Using molecular tools and mathematical modelling, we aimed to elucidate the relative extent of zoonotic transmission from various animal reservoirs, and how this may vary between contrasting geographic regions. *S. japonicum* larvae from a range of definitive host species in hilly and marshland regions of Anhui

Province, China, and in Samar Province, the Philippines, were genotyped using microsatellite markers. In the Philippines, there was no evidence of genetic structuring across host species, suggesting a high frequency transmission across host species, and that dogs could be very important reservoirs in this setting. In China, strong genetic differentiation was observed between *S. japonicum* populations from hilly and marshland regions, and phylogenetic analyses showed substantial clustering of isolates by host species in sympatric villages, suggesting some degree of differential transmission among species. However, within most Chinese villages genetic differentiation among host species was low suggesting frequent parasite gene-flow, and thus also transmission, across species, with rodents potentially acting as the main reservoir hosts in hilly regions, in contrast to bovines in the marshland regions. Results from a multi-host transmission model, fitted to parasitological data collected in Anhui, support this hypothesis, and suggest that current levels of infection among humans in this region of China are not sufficient by themselves to maintain endemic transmission, and may in fact largely result from transmission spillover from reservoir populations. The relative importance of different reservoir host species for *S. japonicum* appears to vary greatly from region to region, and targeted management of transmission within zoonotic reservoirs may be crucial if goals to further reduce the number of human cases, and potentially eliminate *S. japonicum*, are to be achieved.

330

IMPACT OF INTENSE, LONGITUDINAL RETREATMENT WITH PRAZIQUANTEL ON CURE RATES OF SCHISTOSOMIASIS MANSONI IN A COHORT OF OCCUPATIONALLY EXPOSED ADULTS IN WESTERN KENYA

Carla L. Black[1], Michelle L. Steinauer[2], Pauline N. Mwinzi[3], W. Evan Secor[4], Diana M. Karanja[3], Daniel G. Colley[1]

[1]University of Georgia, Athens, GA, United States, [2]University of New Mexico, Albuquerque, NM, United States, [3]Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya, [4]Centers for Disease Control and Prevention, Atlanta, GA, United States

As current schistosomiasis control strategies are focused on mass drug administration with praziquantel (PZQ), drug resistance is a major concern. We report on the efficacy of PZQ after 12 years of intense use in a small geographic area along the shores of Lake Victoria in which men (N=174) occupationally exposed to *Schistosoma mansoni* were repeatedly retreated as part of a longitudinal study. Overall, the cure rate after a single dose of PZQ was 66%, ranging annually from 36% to 82%. In multivariate analysis, failure to cure after 1 PZQ dose was significantly associated with high intensity of infection (OR=1.5 [95% CI 1.2, 1.7] for each 1-unit increase in log egg count) and being treatment naïve (OR=2.5 [95% CI 1.3, 4.7] relative to persons with 3 or more previous cures). Even after adjustment for these factors, treatments administered in the years 2002 and 2006-2007 were significantly more likely to result in cure failures than treatments administered in 2004, the year in which PZQ efficacy was highest. In 2006, the year in which relative cure rates were lowest, the odds of cure failure were 5.8 times greater (95 % CI 2.9, 11.6) than the odds of cure failure in 2004. However, while cure rates varied over the course of 12 years, there was no consistent downward trend towards decreased efficacy over time. In years for which malacological data were available, periods of low PZQ efficacy coincide with high rates of *S. mansoni* infection in snail populations in the area. The percentage of snails infected with *S. mansoni* was 22% in mid-2006, compared to 0-4% in 2004-2005. Because of the increase in the proportion of infected snails, schistosome transmission was likely higher during these times, consistent with the possibility that subjects were harboring pre-patent infections against which PZQ is ineffective. We did not, in our intensely treated cohort, find a pattern of cure failures consistent with development of clinical resistance to PZQ. However, neither did we find that repeated treatment of these men led to decreased transmission of *S. mansoni* in this location.

331

RELATIONSHIP BETWEEN MATERNAL ANEMIA OF INFLAMMATION AND BIRTH OUTCOMES IN *SCHOSTOSOMA JAPONICUM* ENDEMIC VILLAGES OF LEYTE, THE PHILIPPINES

Jennifer F. Friedman[1], Luz P. Acosta[2], Mario A. Jiz[1], Blanca Jarilla[2], David Margolius[1], Courtney Olson[1], Mary Paz Urbina[2], Remigio M. Olveda[2], Jonathan D. Kurtis[1]

[1]Center for International Health Research, Lifespan Hospital/Brown University, Providence, RI, United States, [2]Research Institute of Tropical Medicine, Manila, Philippines

Although maternal anemia is related to low birth weight, the mechanisms through which this occurs are unclear. The most common cause of maternal anemia is iron deficiency (IDA), yet most trials providing iron supplementation during pregnancy have not demonstrated improved birth outcomes. The objectives of this study were to examine 1) the types of anemia among pregnant women in Leyte, The Philippines, where *Schisosoma japonicum* is endemic, 2) the relationship between *S. japonicum* and non-iron deficiency anemia (NIDA), and 3) the relationship between the presence of maternal NIDA and birth outcomes (birth weight and iron status). We enrolled 149 women during the second trimester of pregnancy. At enrollment, the following data were collected: pre-natal history, SES, height, weight, and smoking status. Three stools were collected and examined in duplicate to quantify the intensity of infection with *S. japonicum* and geo-helminths. At 32 weeks gestation, we collected a maternal blood sample to assess complete blood count, as well as markers of inflammation and iron status. This included hepcidin, the putative link between inflammation and anemia. At delivery, the newborn was weighed and a cord blood sample was taken and also assessed for markers of inflammation and iron status. IDA was defined as hemoglobin < 11.0 g/dl and serum ferritin < 30 ng/ml and NIDA was defined as hemoglobin < 11.0 g/dl and serum ferritin > 30 ng/ml. In this population, NIDA is overwhelmingly due to anemia of inflammation (AI). Overall, 66% of women were anemic; 57% had iron deficiency anemia and 9% had NIDA. As previously demonstrated in non-pregnant subjects, women with increasing intensities of *S. japonicum* infection were more likely to have NIDA ($P < 0.03$), but not IDA after adjusting for confounders in multivariate models. *S. japonicum* uninfected women had an adjusted probability of NIDA of about 7% versus 20% among women with moderate intensity. The presence of NIDA, but not IDA, was related to decreased adjusted birth weight with NIDA women having birthweights 346 grams lower than women without NIDA ($P < 0.01$). Finally, newborn ferritin levels were inversely related to maternal levels of hepcidin. This study suggests an important role for AI in mediating adverse birth outcomes. Iron supplementation in the context of inflammation may not demonstrate improved birth weight due to alterations in iron absorption and metabolism, decreasing iron bioavailability in this context.

332

ESTIMATION OF ATTRIBUTABLE RISK OF ANEMIA DUE TO SCHISTOSOMIASIS IN WESTERN KENYA

Susan P. Montgomery[1], Erick M. Muok[2], Pauline N. Mwinzi[2], John M. Williamson[1], W. Evan Secor[1], Diana M. Karanja[2]

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

Subtle morbidities associated with schistosomiasis in schoolchildren can adversely affect physical and cognitive development. Accurate assessment of subtle morbidities is needed to design efficient control programs and better evaluate their impact. To this end, we attempted to estimate the fraction of anemia attributable to schistosomiasis in an area endemic for other anemia-inducing infections and where causes of anemia are likely multifactorial. Children aged 9 - 12 years and attending public schools within 6 km of Lake Victoria in Asembo, Kenya were eligible for participation. Stool and fingerstick blood samples were collected to assess parasite infection status and hemoglobin levels. Socioeconomic

and demographic data were collected and location of schools relative to the lake edge was determined. We used a multivariate modeling strategy (Bruzzi, 1985) to estimate attributable risk of anemia due to schistosomiasis. A total of 2826 children were enrolled. Of these, 2750 provided stool samples; 460 (17%) were positive for *Schistosoma mansoni.* In addition, 176 (7%) were positive for ascarid, 855 (31%) for hookworm and 339 (12%) for trichurid eggs. Of 2701 blood samples collected, 1472 (55%) were positive for *Plasmodium falciparum*. In 95 (6%) children, coinfection with *P. malariae* and *P. ovale* was identified. Anemia (hemoglobin < 12 g/dL) was present in 1388 (51%) participants and anemia was moderate or severe (hemoglobin < 7 g/dL) in 89 (3%) of the children. Schistosomiasis (OR 1.3, 95% CI: 1.03 - 1.63), malaria (OR 1.5, 95% CI: 1.27 - 1.77), and age (OR 0.9, 95% CI: 0.86 - 0.97) significantly contributed to anemia (p <.05) when controlling for other infections, body mass index, and socioeconomic status. The population attributable risk of anemia due to schistosomiasis was 4.0%; attributable risk due to malaria was 19.7%. This study demonstrates the complicated realities of morbidity assessment in the presence of multiple infections and suggests that the fraction of anemia caused by schistosomiasis is less than 10% in this setting of relatively low schistosomiasis prevalence.

333

SCHISTOSOMIASIS AMONG YOUNG CHILDREN IN WESTERN KENYA

Jennifer R. Verani[1], Bernard Abudho[2], Susan P. Montgomery[1], Pauline M. Mwinzi[2], Hillary L. Shane[1], Sara E. Butler[1], Diana M. Karanja[2], William E. Secor[1]

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

Although schistosomiasis burden is greatest among school age children (SAC) (6-15 years), studies using sensitive diagnostic methods have found substantial rates of infection among pre school age children (PSAC) (1-5 years). PSAC are typically not targeted by mass drug treatment for schistosomiasis, yet may be highly susceptible to schistosomiasis-related morbidities such as anemia, growth and cognitive delays. We hypothesize that infected PSAC may excrete fewer eggs in stool than infected SAC, resulting in false negative stool exams and an underestimation of schistosomiasis burden for PSAC. We conducted a cross sectional study evaluating the sensitivity of stool exam for diagnosing *Schistosoma mansoni* infection among children aged 1-15 years in a highly endemic village in western Kenya. Three stools from each study participant were collected; duplicate slides of each sample were examined using Kato/Katz technique. A child with one or more positive slides was considered stool-positive. Serum was collected for anti-schistosome antibody testing to compare to stool results. A total of 249 SAC and 196 PSAC were enrolled; 140 (56%) SAC and 46 (23%) PSAC were stool-positive for schistosomiasis. Prevalence increased with age, with 10% of 1 year olds and 85% of 15 year olds infected. A single stool exam detected 176 (95%) of all 186 stool-positive children; the sensitivity of a single stool exam was significantly lower among PSAC (87%) than among SAC (97%), p=0.016. In conclusion, most schistosomiasis control programs are based on mass treatment of SAC. However, the prevalence among PSAC was much higher than expected and argues that control programs in some areas should include this younger age group. Single stool exam may underestimate the schistosomiasis prevalence, particularly among PSAC. Pending serology results will be used to evaluate the sensitivity of stool exam for diagnosing infection among PSAC vs. SAC, and may reveal an even higher burden among PSAC.

334

MATHEMATICAL MODELS FOR SCHISTOSOMIASIS TRANSMISSION DYNAMICS AND CONTROL IN SUB-SAHARAN AFRICA: LESSONS FROM KENYA AND UGANDA

Michael D. French[1], Thomas S. Churcher[2], Jimmy Kihara[3], Joanne P. Webster[1], Maria-Gloria Basáñez[2]

[1]Schistosomiasis Control Initiative, Imperial College London, London, United Kingdom, [2]Department of Infectious Disease Epidemiology, Imperial College London, London, United Kingdom, [3]Kenya Medical Research Institute (KEMRI), Nairobi, Kenya

Large-scale control programs, such as the Schistosomiasis Control Initiative (SCI) and others in Sub-Saharan Africa, are providing an invaluable opportunity to parameterize mathematical models in specific settings with important theoretical and applied implications. Age-structured mathematical models such as EpiSchisto® were fitted by maximum likelihood to detailed (mean and individual) data from Kenya and Uganda, describing age infection profiles and temporal dynamics of infection after various treatment rounds with praziquantel, taking into account overdispersion in egg counts per gram of feces (epg). In Kenya, the importance of micro-epidemiology in schistosomiasis transmission was highlighted by the need to fit models to irrigated versus non-irrigated areas separately. In Uganda models were fitted to areas with low, moderate, and intense transmission. In low transmission areas, reducing treatment frequency from once per year to every other year may be considered as an effective option in resource-constrained settings, whilst still protecting reductions in morbidity that have been achieved. The relationship between infection prevalence and intensity was analyzed for several rounds of treatment in order to investigate changes in the degree of parasite overdispersion under chemotherapy. It was established that although the degree of overdispersion is a function of infection intensity (as measured by epg), a single relationship encapsulating those changes can be usefully incorporated into mathematical models. We will discuss the effect of varying degrees of density-dependent parasite fecundity (as estimated from different -observational and experimental- approaches) on the rates of parasite reinfection after chemotherapy. We highlight the need to use robust mathematical approaches along with monitoring and evaluation of control programs and the importance of fitting models to post-treatment data to identify the effects of anthelmintic treatment on macroparasite population biology.

335

INTEGRATING PROTOCOLS FOR MAPPING TRACHOMA AND URINARY SCHISTOSOMIASIS. CAN SURVEYS BE DONE SIMULTANEOUSLY?

Jonathan D. King[1], Frank Richards[1], Abel Eigege[2], Nimzing Jip[2], John Umaru[2], Michael Deming[3], Deborah McFarland[4], Emmanuel Miri[2], Paul M. Emerson[1]

[1]The Carter Center, Atlanta, GA, United States, [2]The Carter Center, Jos, Nigeria, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States, [4]Emory University, Atlanta, GA, United States

Integrated approaches to neglected tropical disease control are being strongly promoted. Disease mapping is a key component in directing control activities. The purpose of this study was to determine whether the school-based methodology used to measure the prevalence of hematuria as a biomarker for urinary schistosomiasis (SCH) would be useful for determining trachoma prevalence, and whether the district-level approach recommended for trachoma would be useful for SCH control programs. We conducted two separate integrated surveys in 8 districts of Plateau and Nasarawa States, Nigeria: school-based and district-based. For the school-based survey, we visited all rural primary schools in the 8 districts, taking a systematic sample of 32 to 47 children per school for each disease. For the district-based survey, we visited all households in a randomly-chosen segment within each of 20 enumeration areas (clusters) per district and screened all people for trachoma and children for SCH. Clinical signs of

trachoma were assessed using the WHO Simplified Grading System and hematuria was detected by a dipstick test. A total of 16,991 children were examined for trachoma and 14,003 children were examined for hematuria from 363 schools and 2,121 households. District-level estimates for active trachoma from both surveys were less than 10% in children 1-9 years-old for all districts (the threshold criterion for trachoma interventions including mass antibiotic treatment). From the school survey results, communities surrounding 142 schools warrant trachoma control activities of which 53 qualify for mass antibiotic distribution. Communities of 67 schools warrant drug treatment for SCH. District estimates of the prevalence of hematuria from trachoma survey methodology ranged from 1.21% to 19.4%. Using these estimates, only one district qualified for drug treatment, missing 49 communities qualified for treatment of school-aged children and 8 communities qualified for mass treatment. Integrating trachoma into SCH school surveys, and SCH into trachoma surveys, was quick and easy. School-based surveys were useful in this hypo-endemic area for identifying communities in which trachoma intervention is warranted. However, district-level estimates of SCH from integrated cluster surveys in this study were not useful for planning treatment interventions and confirm the need for a community-based approach to mapping SCH.

336

EXPERIMENTAL MALARIA INFECTION TRIGGERS RAPID EXPANSION OF NATURAL KILLER CELLS

Sunil Parikh, Charlie C. Kim, Joseph C. Sun, Alissa Myrick, Lewis L. Lanier, Philip J. Rosenthal, Joseph L. DeRisi

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

To gain a better understanding of gene expression during early malaria infection, we conducted microarray analysis of early blood responses in mice infected with erythrocytic stage *Plasmodium chabaudi*. Blood from C57BL/6 mice was harvested every 8 hours after intraperitoneal infection with 10^6 parasites. Two aspects of the immune response were particularly striking. First, the response to infection was rapid, exhibiting significant changes in transcript abundance between 8 and 16 hours post-infection. Second, the response occurred in coordinated waves of expression. Immediately following infection, we observed coordinated and sequential waves of immune responses, with interferon-associated gene transcripts dominating by 16 hours post-infection, followed by strong increases in natural killer (NK) cell-associated and MHC class I-related transcripts by 32 hours post-infection. We hypothesize that the observed elevation in NK cell-associated transcripts is the result of a dramatic increase in the proportion of NK cells in the blood during infection. Analysis of the major leukocyte components of the blood and spleen by FACS revealed a 5-10 fold increase in NK frequency in the peripheral blood. We then compared expression profiles of NK cells isolated from the blood of mock-infected versus *P. chabaudi*-infected mice at 72 h post-infection. Comparative analysis showed that the increased abundance of NK-associated lectin-like killer cell receptor family transcripts observed in whole blood at 32 h was primarily due to an increase in NK cell frequency rather than a transcriptional response to infection. Intriguingly, there were few signatures which suggested that the NK cells might be activated, yet key indicators of a proliferative response were upregulated. These findings were confirmed by early and rapid proliferation of NK cells in studies using adoptively transferred carboxyfluorescein diacetate, succinimidyl ester-labeled cells in infected mice. Our results demonstrate that rapid immune responses occur in the blood and spleen in response to P. chabaudi infection. Specifically, an early interferon response is followed by a dramatic increase in circulating NK cells, which is at least partially explained by the observation that these cells are undergoing replication.

337

SERUM VON WILLEBRAND FACTOR LEVELS EFFECTIVELY DISCRIMINATE BETWEEN CEREBRAL MALARIA AND UNCOMPLICATED MALARIA

Gregory S. Park[1], Robert O. Opoka[2], Michael J. Boivin[3], Chandy C. John[1]

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

More than 500 children are admitted to Mulago Hospital in Kampala, Uganda yearly with *P. falciparum* cerebral malaria (CM), and 16.8% of cases are fatal. Endothelial cell (EC) activation appears to play a role in CM pathogenesis, but plasma levels of EC activation markers and their relationships to malaria pathogenesis have not been fully characterized. We measured von Willebrand factor (vWF), vascular cell adhesion molecule-1 (sVCAM-1), and intercellular adhesion molecule-1 (sICAM-1) levels in the sera of 75 Ugandan children with CM, 65 children with uncomplicated malaria (UM), and 49 healthy community control (CC) children. vWF levels were significantly higher in children with CM than UM (P<0.0001) and in children with UM than CC (P<0.0001). In contrast, sVCAM-1 and sICAM-1 concentrations were marginally higher in children with CM than UM (sVCAM-1, P=0.03; sICAM-1, P=0.12), but significantly higher in children with UM than CC (sVCAM-1, P<0.0001; sICAM-1, P<0.0001). Levels of all three factors correlated with each other (r=0.401 - 0.645, P<0.0001). To determine which marker best differentiated CM and UM, receiver operating characteristic (ROC) analyses were performed. Results indicated that only vWF levels effectively distinguished between CM and UM groups (AUC=0.725, 95%CI 0.641, 0.810). These data suggest that in Ugandan children, cerebral malaria is associated with greater EC activation than uncomplicated malaria. vWF levels may be the best marker of EC activation in these children.

338

B CELL ACTIVITY IN CHILDREN WITH MALARIA

Jackson C. Korir[1], Ronald P. Taylor[2], John N. Waitumbi[1]

[1]Walter Reed Project/KEMRI, Kisumu, Kenya, [2]Department of Biochemistry and Molecular Genetics, University of Virginia School of Medicine, Charlottesville, VA, United States

Mature B cells express CD20 molecules on their surface that distinguishes them from antibody-producing plasma cells. The antigen-processing and presentation capabilities of mature B cells are enhanced by their ability to bind complement-opsonized immune complexes (IC) via the membrane bound receptor CD21 (CR2). B-cell numbers and expression levels of both cell-associated and soluble CD21 (sCD21) are affected differently by different diseases and this has impact on the competency of B cells. In a case control study, we assessed how malaria affects B cell numbers and expression levels of CD21 in children presenting at Kisumu District Hospital, western Kenya with either severe malarial anemia (SMA) or uncomplicated malaria. Children with SMA had a higher % of CD20 B cells (26.8±9.7 S.D) as compared to their age and sex matched controls (20.9±9.0 S.D, P=0.025) probably as a result of polyclonal activation of B cells by malarial antigens. However, the median fluorescence intensity for CD21 on the mature B-cells of children with SMA was much lower (251.9±90.29 S.D) than that of the controls (372.99±140.82, P=0.01). We think this results from the processing and removal of CD21, along with associated complement-opsonized IC, by fixed tissue macrophages of the mononuclear phagocytic system (MPS). In addition, the B-cells of SMAs had higher levels of the complement split product C3dg (18.91±11.66 S.D) compared to controls (11.5±7.33 S.D, P=0.02), pointing to increased complement activation in the SMA group, a phenomenon that has been observed in severe malarial anemia. We also found that children with SMA had lower levels of sCD21 (223.70±131.79 S.D) compared to controls (341.43±137.32 S.D, P=0.003). This result indicates that, unlike in the normal enzymatic cleavage of B cell-associated CD21 and subsequent