selected compound then co-infused in the microfluidic device, in mice or in the human spleens along with the same gametocyte population exposed to the solvent control. Normal RBCs, asexual ring-IRBCs and heated RBCs were used as controls. We first confirmed that unlike stage I-IV, stage V gametocytes from an *in vitro* culture were not markedly retained in microsphere-based microplate filters and in human spleen perfused ex-vivo, consistent with the hypothesis that deformability of gametocytes is a major determinant of their circulation in peripheral vessels. Using the microfluidic device, we showed that stage V exposed to a recently identified stiffening compound C were enriched to 74.9% (vs. 25.08% for unexposed controls, p=0.0001 paired t test) in narrow 2 µm-wide spaces mimicking inter-endothelial slits in the spleen. In macrophage-depleted C57 Bl/6 mice, immature gametocytes (10 mice) and heated RBCs (4 mice) were cleared by 86% or 75% in 3 hours, respectively. By contrast, a majority of mature gametocytes (5 mice) or normal RBCs (4 mice) were still circulating 3 hours after infusion (Retention rates: 44% and 30%, respectively (p=0.0058, p=0.0002). Similar results were observed in human spleens. Mature circulating gametocytes can be stiffened to induce their mechanical retention, thereby interrupting transmission. The stiffening effect can now be validated in a biomimetic microfluidic device and in a simple rodent model as a prerequisite before further development.

1499

THE USE OF RESPONDENT DRIVEN SAMPLING METHODS TO IDENTIFY MALARIA PREVENTION KNOWLEDGE AND BEHAVIORS BY MIGRANT AND MOBILE POPULATIONS IN WESTERN CAMBODIA

John Hustedt[1], Chandary Rang[1], Sophal Uth[1], Sara Canavati[1], John Macom[1], Po Ly[2], Siv Sovannaroth[2], Arantxa Roca-Feltrer[1], Tarekegn Abeku[1], Sylvia Meek[1]

[1]Malaria Consortium, Phnom Penh, Cambodia, [2]National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia

Mobile and migrant populations (MMPs) along the Thai-Cambodian border are at high-risk for malaria infection and have been found with artermisinin resistant parasites. However, the mobile nature of this population makes it difficult to adequately measure malaria infection and risk behaviors, which is vital as we move to elimination in the region. Utilizing respondent driven sampling methods, MMPs residing within two villages in Palin province (Pang Rolim and Sala Krau) were recruited in two independent rounds of sampling (602 in 2013 and 604 in 2014). All responses were adjusted for network size and recruitment patterns allowing for calculation of population-adjusted statistics. While the prevalence of *Plasmodium vivax* is estimated to be 0.2% among the general population, this study found 2.0% and 1.3% of MMPs in these networks to be infected with *P. vivax* in 2013 and 2014 respectively, and an absence of *P. falciparum*. Most respondents from Pang Rolim, from both rounds, identified having seen malaria messages within the previous three months (99.7%, 95% CI: 97.6-100 in 2013 and 99.0%, 95% CI: 95.9-99.8 in 2014). However, in Sala Krau, the percentage of respondents answering similarly decreased from 97.0% (95% CI: 94.1-98.4) in 2013 to 59.1% (95% CI: 51.3-66.4) in 2014. While knowledge related to malaria transmission, symptoms and prevention increased noticeably in Pang Rolim, similar knowledge remained low in Sala Krau across both rounds. Furthermore, while the percentage of respondents from Pang Rolim who didn't use a net the previous night remained the same across both rounds (2.4%), there was a slight increase in non-users in Sala Krau from 6.1% (95% CI: 0.9-6.7) to 9.5% (95% CI: 5.3-16.4). These findings correlate with the fact that there were increased efforts on malaria prevention in Pang Rolim (eg. concerts and videos with prevention messaging) and not in Sala Krau; suggesting that as MMPs change frequently there is a need for sustained public health efforts to reach this population, especially within an elimination context.

1500

IMPLEMENTING ENHANCED HIGH-RESOLUTION SURVEILLANCE USING SPATIAL DECISION SUPPORT SYSTEMS TO GUIDE TARGETED RAPID RESPONSE IN MULTI-DRUG RESISTANT AREAS OF VIETNAM

Gerard C. Kelly[1], Colin Ohrt[1], Hieu M. Truong[2], Hien T. Nguyen[3], Nicholas Martin[4], Xa X. Nguyen[2], Quy Anh Nguyen[2], Roland Gosling[1], Jennifer Wegbreit[1], Duong T. Tran[2], Duong T. Tran[2], Thang D. Ngo[2]

[1]University of California San Francisco, San Francisco, CA, United States, [2]National Institute of Malariology, Parasitology and Entomology, Hanoi, Vietnam, [3]U.S. Embassy, Hanoi, Vietnam, [4]Naval Medical Research Center-Asia, Singapore, Singapore

Emerging artemisinin resistant malaria in the Greater Mekong Subregion (GMS) has important implications for public health. A project was established to research, develop and implement enhanced surveillance and targeted appropriate intervention measures to stop the spread of multi-drug resistant malaria through elimination of the disease in the region. The aims of this project are to pilot a spatial decision support system (SDSS) approach to conduct high-resolution surveillance to guide swift and targeted responses. Pilot sites were established in selected communes in Vietnam with associated customised SDSS developed. Publically available topographic geographic information system data were uploaded into the SDSS to provide baseline information. Household and forest transmission location data were located and enumerated through field-based geographical reconnaissance using handheld computers. Passively detected malaria cases were geo-referenced to the suspected transmission location sites upon diagnosis. Using case location data in the SDSS, active transmission foci were automatically classified and response areas-of-interest (AOI) generated. Supporting data (including population, location and number of sleeping locations within the AOI) were automatically produced in the SDSS and sent to village health workers and district level units to mobilize appropriate responses. Complete pilot data for presentation are expected in September 2014. This new approach utilizing novel geo-spatial tools to support targeted, appropriate and aggressive response measures to support malaria elimination in areas of global significance will be presented.

1501

RESTRATIFICATION OF MALARIA EPIDEMIOLOGY IN VIETNAM FOR MORE EFFECTIVE APPLICATION OF LIMITED RESOURCES

Thang D. Ngo[1], Hung X. Le[1], Hung M. Nguyen[1], Thieu Q. Nguyen[1], Trung D. Ho[1], Xa X. Nguyen[1], Anh Q. Nguyen[1], Ha S. Dinh[1], Roland Gosling[2], Bruce Lee[3], Hien T. Nguyen[4], Gerard C. Kelly[3], Nicholas Martin[5], Colin Ohrt[3], Duong T. Tran[1], The NIMPE Advisory Groups[1]

[1]National Institute of Malariology, Parasitology and Entomology, Hanoi, Viet Nam, [2]University of California San Francisco, San Francisco, CA, United States, [3]U.S. Embassy, Hanoi, Viet Nam, [4]Naval Medical Research Centre - Asia, Singapore, Singapore

The National Malaria Control Program in Vietnam is updating malaria epidemiology in order to more effectively apply limited malaria diagnosis, prevention and treatment resources. The most recent prior restratification was conducted in 2009. This on-going 2014 restratificaton effort (2009-2013 data) is using the same methods of 2009 to collect all malaria case data to the commune (county) level. Indicators for classification are based on the average number of confirmed cases per 1000 population over the 5 year period, the presence of at least one of the three malaria vectors, socioeconomic disadvantaged or border commune, poor health system, drug resistant parasites, chemically resistant mosquitoes, and migratory populations. Each indicator has a score, with the sum of the scores used to define the level of endemicity and priority for interventions. This score will be used to characterize each commune into one of five zones (no

malaria transmission, area at risk for reintroduction of malaria, low (>0-1/1000), medium (1-5/1000), or high (>5/1000)). The current levels of malaria endemicity using historical passive case detection data will be determined by September 2014. Using available data, greater precision of where malaria transmission is occurring and populations at risk will also be estimated. Enhanced methods to collect these data prospectively will be developed. Additionally, data on all prior malaria interventions for the last 5 years will also be collected and entered in to a database. These will be analyzed to estimate the impact of prior malaria control interventions in an operations research model to help select which methods should be continued or reassessed. Methods to prospectively assess the impact of new interventions in an on-going and iterative fashion will also be developed. We will present the new 2014 restratification data and compare and contrast it with the 2009 data. We will illustrate how these new data will be used to better target interventions. The plan to collect more precise prospective information, as well as the status of the analysis of intervention impact, will also be presented.

1502

LAMP AS DIAGNOSTIC TOOL FOR DETECTION OF SUB-PATENT ASYMPTOMATIC MALARIA INFECTIONS IN PRE-ELIMINATION SETTINGS IN NORTHERN NAMIBIA

Davis R. Mumbengegwi[1], Munyaradzi Tambo[1], Ronnie A. Bock[1], Roland D. Gosling[2], Hugh J. Sturrock[2]

[1]University of Namibia, Windhoek, Namibia, [2]University of California San Francisco, San Francisco, CA, United States

The global map of malaria is shrinking with 34 out of 99 malaria endemic countries embarking on eliminating the disease. Transmission in Namibia has declined dramatically from 477,786 cases in 2000 to 1546 in the 2013 malaria season. Namibia is now in the pre-elimination phase of malaria and is targeting elimination by 2020. A new challenge facing the elimination campaign is the detection of asymptomatic malaria cases with low levels of parasitaemia. These infections are difficult to detect as they are below the threshold of routinely used Rapid Diagnostic Test (RDT) kits, yet can result in onward transmission. Molecular tools based on DNA amplification such as PCR are sensitive and specific enough to detect low parasitaemia but their routine use requires expensive, highly technical equipment and expertise. Loop-mediated isothermal amplification (LAMP) is a tool based on DNA amplification and has the advantages of PCR yet is requires less expertise and equipment. This study was conducted to determine the usefulness of LAMP as a diagnostic tool to detect asymptomatic, sub-patent infections found during reactive case detection in Engela district in Northern Namibia. All RDT confirmed malaria cases reported in the Engela district and members of their households as well as occupants of the four surrounding households were recruited into the study. RDTs and dried blood spots (DBS) of all subjects were collected and DNA was extracted from both using the chelex method. LAMP was run using DNA extracted from all the collected samples and results detected as fluorescence under a UV light. Preliminary results from 416 RDTs and DBS collected during follow up of 11 index cases, showed 11 individuals positive by RDT and 18 positive by LAMP. Thus 7 additional secondary malaria cases associated with index cases (a 1.6 fold increase) were detected by LAMP over RDTs. This shows LAMP could be a useful tool to detect sub-patent asymptomatic malaria infections at low transmission and may be a suitable diagnostic tool for use in pre-elimination settings.

1503

BASELINE EPIDEMIOLOGICAL CHARACTERISTICS OF PARTICIPANTS ENROLLED IN A TRIAL OF INTERMITTENT MASS SCREENING AND TREATMENT FOR MALARIA IN WESTERN KENYA

Aaron M. Samuels[1], Norbert A. Odero[2], Wycliffe Onyango[2], Kephas Otieno[2], Peter Otieno[2], Ya Ping Shi[1], Mary J. Hamel[1], Kim Lindblade[1], Simon Kariuki[2], Meghna Desai[1]

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

The prevalence of malaria infection remains high in western Kenya despite over 30 years of control efforts. Community parasite prevalence in children <5 years of age was estimated at >80% in the early 1980s, and dropped to 26% by 2008. Prevalence rose to 43% in 2011. Individuals with asymptomatic parasitemia account for 90% of infections in specific age groups and may be sustaining the continued high level of transmission. Mathematical models suggest that strategies targeting the asymptomatic population could reduce malaria transmission. We have initiated a multi-year two-arm cluster randomized controlled trial to evaluate the impact of intermittent mass screen and treat (iMSaT) campaigns for malaria in Siaya County, western Kenya. We describe baseline cross-sectional survey results. Twenty compounds were randomly selected from each of 20 clusters during peak malaria transmission season in July-August, 2013. All consenting individuals within houses of selected compounds were asked demographic, behavioral and symptom-based questions by community health workers using personal digital assistants. Blood samples were tested for malaria using a combination HRP2/pLDH rapid diagnostic test (RDT), light microscopy (LM), and polymerase chain reaction (PCR). A total of 1,987 persons living in 605 households from 359 selected compounds were interviewed. Of these, 1,402 consented for both RDTs and LM. Baseline malaria infection prevalence was 47.1% (95% Confidence interval [CI] 43.9-50.2) and 36.6% (CI: 33.3-39.8) by RDT and LM, respectively. RDT positivity was strongly associated with age; 64.9% (CI: 57.6-72.2), 70.8% (CI: 65.1-76.4), and 27.8% (CI: 24.5-31.1) of persons aged <5 years, 5-15 years, and older than 15 years were RDT positive ($P < 0.0001$), respectively. Only 25% who were RDT positive reported a fever in the prior 24 hours, and 45% reported a fever in the previous two weeks. Overall, history of fever in the previous 2 weeks was not associated with RDT positivity, PR 0.94 (CI: 0.84-1.04). Of persons reporting a fever in the previous 2 weeks, RDT positive individuals were as likely to seek care as those who were not, PR 1.04 (CI: 0.93-1.16). PCR results and multivariable analyses are pending. The large proportion of infections that were not associated with fever or care-seeking behavior suggests that strategies targeting the asymptomatic population may be beneficial for reducing malaria transmission in western Kenya.

1504

COMMUNITY-LEVEL MALARIA SURVEILLANCE IN SOUTHERN PROVINCE, ZAMBIA - AN ANALYSIS OF PERCEPTIONS, PRACTICE AND PROGRESS IN AN AREA TARGETING ELIMINATION

Lynne Lohfeld[1], Tokozile Kangome-Ngwenya[2], Anna M. Winters[2], Zunda Chisha[2], Busiku Hamainza[3], Mulakwa Kamuliwo[3], John Miller[4], Matthew Burns[2], Daniel J. Bridges[2]

[1]McMaster University, Hamilton, ON, Canada, [2]Akros, Lusaka, Zambia, [3]Zambia National Malaria Control Center, Lusaka, Zambia, [4]PATH-MACEPA, Lusaka, Zambia

Since its inception in 2011, the training techniques and personnel associated with the program have evolved and matured using anecdotal feedback from community health workers (CHWs) trained to deliver the program, their supervisors and district-level staff. We have recently conducted a systematic qualitative program review to determine how malaria is perceived by the program implementers and beneficiaries, how the community received the surveillance platform, and solutions to key

operational challenges that could improve implementation. A criterion-based sampling framework based on training regime and performance level of facilities offering the program was used to select six rural health posts, three per district, and a representative from associated stakeholder groups (community health workers delivering the program, their clinic-based supervisors, regional-level staff, and community members). Individual interviews and focus group discussions were then held in these selected sites. Service providers, supervisors, program administrators and community members all credited the program with helping to reduce the number of malaria cases. Barriers to fuller implementation of the program included transportation (e.g. ensuring all CHWs had working bicycles), communication (e.g. providing CHWs with working cell phones and "talk time" to transmit data by phone) and supplies (e.g. ensuring adequate number of RDT kits to test for malaria in clinics and communities, artemether-lumefantrine to treat uncomplicated malaria cases, and antipyretics for malaria-negative patients to encourage future visits to rural health centers). Results from this review will be used when developing plans to scale-up the program for delivery in other parts of Zambia.

1505

REACHING MIGRANT AND MOBILE POPULATIONS THROUGH A PRIVATE SECTOR INITIATIVE: MALARIA BED NET LENDING SCHEME IN CAMBODIA

Soy Ty Kheang

University Research Co. LLC, Phnom Penh, Cambodia

While tremendous strides have been made toward eliminating malaria in Cambodia, pockets of risk persist in border areas that are remote and have high population mobility. Lacking knowledge of malaria, migrant and mobile populations (MMPs) are particularly at risk. The PMI/USAID Control and Prevention of Malaria (CAP-Malaria) Project in the Greater Mekong Sub-region works with private sector employers to increase access to and use of long-lasting insecticidal nets (LLINs) by their migrant workers. Employers range from small farm owners to large companies that manage rubber, cassava plantations, and hydroelectric dam construction. Employers receive a stock of LLINs and malaria educational materials for their employees. The employers then lend the LLINs to their workers, retrieving them prior to their departure for reuse with other migrant employees. An evaluation of the lending model was conducted during the harvesting season in late 2013. The study assessed access to and utilization of LLINs by migrant workers, and explored reasons for non-use. Interviews were conducted with 207 farm owners and 712 workers. Results showed that farm owners were generally satisfied with the LLIN lending model. Some employers (28%) ran out of nets. LLIN uptake among the workers was high, most (93%) had a bed net at their residence, and almost all (96%) reported sleeping under a bed net the previous night. Half of the workers (58%) had received an LLIN from their employer. The main barrier for not using a LLIN was that it was considered too stiff (29%). A fifth of respondents also said they were allergic to the insecticides. Half of the farm workers said they would be willing to pay a small amount for their own net, suggesting an opportunity for subsidized vouchers for LLINs.

1506

MODELING PHARMACOKINETICS AND PHARMACODYNAMICS OF ANTIMALARIAL DRUGS IN THE EPIDEMIOLOGICAL MODELING (EMOD) MODEL WITH IMPLICATIONS FOR TRANSMISSION REDUCTION

Jaline Gerardin, Edward Wenger, Philip Eckhoff

Intellectual Ventures, Bellevue, WA, United States

Modeling approaches can predict the effect of large-scale drug deployments on reducing morbidity over several years of campaigns, leading to better understanding of the role of antimalarial drugs in eradication. The Epidemiological Modeling (EMOD) simulation program includes individual-level modeling of vectors and within-host dynamics, providing an ideal environment in which to study the interplay of antimalarial drugs and host immunity in clearing both asexual parasites and transmission-stage gametocytes. We modeled the pharmacokinetics (PK) and pharmacodynamics of two artemisinin-based combination therapies (ACTs), artemether-lumefantrine (AL) and dihydroartemisinin-piperaquine (DP), and one gametocytocidal drug, primaquine, using age-based dosing and weight-dependent PK. We show that current dosing regimens, especially current fixed-dose recommendations for DP, significantly underdose children. We also find that asexual-stage immunity alone is insufficient to explain low gametocyte prevalence in populations with endemic malaria; host physiological responses are likely to modulate prevalence of the sexual stage. We identify a maximum EIR above which co-dosing ACTs with primaquine has little effect on reducing prevalence, and we demonstrate that a minimal level of individual compliance is necessary for mass drug treatments to impact transmission. Pharmacological modeling of antimalarials can guide community decisions in drug administrations and alert administrators to the most likely and deleterious modes of drug failure.

1507

SIMULATION OF MALARIA PARASITE RESERVOIR COVERAGE USING REACTIVE CASE DETECTION AND ACTIVE COMMUNITY FEVER SCREENING FROM CENSUS DATA IN SOUTHERN ZAMBIA

Joshua O. Yukich[1], Adam Bennett[1], Rudy K. Yukich[2], Busiku Hamainza[3], Kafula Silumbe[4], John M. Miller[4], Thomas P. Eisele[1]

[1]Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States, [2]Sensorstar, Inc., Ellicott City, MD, United States, [3]National Malaria Control Centre, Ministry of Health, Lusaka, Zambia, [4]PATH-MACEPA, Lusaka, Zambia

There is need for malaria elimination programs to determine when and where reactive case detection (RCD) is most effective and feasible. Geo-referenced census data on over 80,000 individuals from 6 rounds of a mass test and treatment (MTAT) intervention with rapid diagnostic tests (RDT) in southern Zambia (2012-2013) were analyzed using a Monte-Carlo simulation algorithm to assess the coverage, sensitivity and specificity of potential RCD systems. Data on household location and composition, fever history, treatment seeking, and RDT results for all individuals were included in MTAT census data. Simulations were conducted within 25 health facility catchments, and the following parameters were varied in sensitivity analysis: RCD search radius or number of households searched, sensitivity and specificity of diagnostics used to identify index cases, treatment seeking probability, household and individual RCD participation level, sensitivity and specificity of diagnostic used during RCD search. Results indicate that RCD and active community fever screening are potentially efficient ways of identifying the parasite reservoir. However, substantial resources are required before meaningful fractions of the parasite reservoir are found in a single search round. Treatment seeking for fevers and access to care are key limiting factors to the sensitivity of an RCD system for identifying the parasite reservoir in the community. A shift from RCD to active community fever screening would improve the fraction of the parasite reservoir identified, especially in areas with poor access to care. However, the fraction of the parasite reservoir identified remains small given feasible search criteria in both systems. Multiple RCD rounds may improve the fraction identified over a given period of time.

1508

MAPPING GLOBAL MALARIA CONNECTIVITY FOR STRATEGIC ELIMINATION PLANNING

Andres Garcia[1], Alessandro Sorichetta[2], David L. Smith[3], Deepa K. Pindolia[4], **Andrew Tatem**[2]

[1]Bill & Melinda Gates Foundation, Seattle, WA, United States, [2]University of Southampton, Southampton, United Kingdom, [3]Johns Hopkins School of Public Health, Baltimore, MD, United States, [4]Clinton Health Access Initiative, Boston, MA, United States

Calls for the eradication of malaria require the development of global and regional strategies based on a strong and consistent evidence base. Evidence from the previous global malaria eradication program and more recent transborder control campaigns have shown the importance of accounting for human movement in introducing infections to areas targeted for elimination. Here, (micro)census, survey and cellphone-based human movement data and models were analysed with network analysis tools to map globally the connectivity of both countries and subnational administrative units through population movements. These data were also combined with *Plasmodium falciparum* and *P. vivax* malaria transmission maps and a global population dataset to identify the likely principal sources and destinations of imported cases. Results indicate that certain groups of countries and regions within countries are much more strongly connected by high levels of population movement than others. The mapping here of both communities of subnational regions and countries linked by high levels of population exchange, and 'natural' migration boundaries that display reduced movement of people and infections between regions has practical utility. These inform the design of malaria elimination strategies by identifying regions afforded protection from re-colonisation by natural 'firebreak' regions of reduced connectivity. For more isolated areas, a regionally-focussed control or elimination program is likely to stand a better chance of success than those receiving high levels of visitors and migrants from high transmission regions. Moreover, we demonstrate how the mobility and malaria connectivity framework provides an evidence base for informing the design and simulation of malaria elimination strategies globally.

1509

PHASE 2A DOSE ESCALATION STUDY OF SAFETY AND EFFICACY OF LOW SINGLE-DOSE PRIMAQUINE FOR GAMETOCYTOCIDAL ACTIVITY AGAINST *P. FALCIPARUM* IN SUB-SAHARAN AFRICA

Halimatou Diawara[1], Joelle Brown[2], Ibrahima Baber[1], Almahamoudou Mahamar[1], Koualy Sanogo[1], Harouna Soumare[1], Fanta Koita[1], Eugenie Poirot[2], Jimee Hwang[2], Sekou Traore[1], Francois Nosten[3], Teun Bousema[4], **Alassane Dicko**[1], Roland Gosling[2]

[1]Malaria Research and Training Center, Bamako, Mali, [2]Global Health Group, University of California San Francisco, San Francisco, CA, United States, [3]Mahidol Oxford University Research Unit in Bangkok, Oxford, United Kingdom, [4]University of Nijmegen, Nijmegen, Netherlands

Primaquine is the only currently available drug with strong gametocytocidal properties against the more mature gametocytes and known to behighly effective in reducing gametocyte carriage and infectivity to mosquitoes. However its deployment hasbeen limited because of the safety concerns. To identify the lowest efficacious dose of PQ, we conducted a Phase 2a dose escalation study of safety and efficacy of low single-dose primaquine in non deficient G6PD male in Ouelessebougou Mali. The first 50 participants aged 5 to 50 years with *Plasmodium falciparum* gametocyte at blood smear, were randomly allocated to one of the following treatment groups with primaquine at 0, 0.125 mg/kg and 0.5 mg/kg. All participants received standard dose dihydroartemisinin-piperaquine. Subjects were seen at days 0, 1, 2, 3, 7, 14 and 28 for hemoglobin measurement and assessment adverse events. Mosquitoes were fed on blood meal using membrane feeding assay before administration of drug and 1, 2 and 7 days after. Infectivity to mosquitoes was measured by the presence of oocysts 7 days post infected feeding. There was no severe or serious adverse event. Preliminary analysis on the first 30 participants enrolled showed no differences among the three groups in mean change in hemoglobin following treatment on day 1 (p=0.89), day 2 (p=0.77), day 3 (p=0.10), day 7 (p=0.61), day 14 (p=0.66), and day 28 (p=0.81). The mean hemoglobin was 13.8 g/dL (range: 11.5, 16.2) at day 0, 13.9 g/dL (range: 11.3, 17.9) at day 7, and 14.0 g/dL (range: 11.6, 17.3) at day 28. In the control group, compared to day 0 there was no reduction in infectivity on day 2 or day 7, -22.8% (95% CI -100%,100%) and 40.0% (95% CI -86%, 100%), respectively. In the 0.125 mg/kg dose group, compared to day 0, there was 86.3% (95% CI 39.4%, 100%) reduction on day2 and 100% reduction in day 7. In the 0.5 mg/kg dose group, compared to day 0 there was a 100% reduction in day 2 and 90.1% (95% CI 64.7%, 100%) reduction on day 7. In summary our preliminary results indicate a higher reduction in infectivity in the 0.5 mg dose group (100% reduction at day 2) without safety concerns.

1510

GENOME-SCALE PROTEIN MICROARRAY ANALYSIS OF *PLASMODIUM FALCIPARUM* AND *P. VIVAX* SYMPTOMATIC AND ASYMPTOMATIC INFECTIONS IN A REMOTE VILLAGE OF THE PERUVIAN AMAZON

Raul Chuquiyauri[1], Douglas Molina[2], Huw Davies[3], Hugo Rodriguez[4], Alejandro Llanos-Cuentas[5], Philip Felgner[3], Joseph M. Vinetz[1]

[1]University of California San Diego, San Diego, CA, United States, [2]Antigen Discovery Inc., Irvine, CA, United States, [3]Division of Infectious Diseases, Department of Medicine, University of California Irvine, Irvine, CA, United States, [4]Directorate of Health, Iquitos, Peru, [5]Tropical Medicine Institute "Alexander von Humboldt", Universidad Peruana Cayetano Heredia, Lima, Peru

The Peruvian Amazon is hypoendemic for *Plasmodium falciparum* (Pf) and *P. vivax* (Pv) malaria. To assess, at a population level, the proportion of people infected by the endemic malaria parasites, a prospective cohort study was done in Santa Emilia, a remote-rural village in the Peruvian Amazon in 2013 where the main seasonality for malaria relates to river height rather than rainfall. Light microscopy and an aldolase gene-specific qPCR assay that detects both Pv and Pf were used to assess the presence of parasitemia. Plasmas were obtained for genome-scale protein microarray analysis using a combined Pf500/Pv500 chip containing a down selected list of the 500 of the top most sero-reactive antigens for each Pf and Pv. At baseline, 33(22%) and 77(51%) of 151 subjects were positive by microscopy and q-PCR, respectively. On the last survey, 3(2%) and 8(5%) of 157 subjects were positive by microscopy and q-PCR, respectively. Asymptomatic parasitemia detected by microscopy ranged from 22% to 40%, while for q-PCR ranged from 41% to 60%. A significant proportion of infections detected by q-PCR, 39% and 47% for Pf and Pv, respectively, were undetected using microscopy. Comparison of proportions of negative subjects at each of the eleven surveys revealed there was a significant increase of negative subjects during September to December surveys in comparison to March surveys. Protein microarray analysis was done with 324 plasma samples: 132 matched paired samples from the two time points and 60 unpaired samples. The top 200 most sero-reactive antigens were selected for comparison. Seroreactivity increased with age and in response to documented malaria infection. Seroreactivity was lower in September than in March for all age groups, paralleling mosquito abundances (related to river height, not rainfall). While there was a gradual increase in seroreactivity associated with age, the differential seroreactivity between March (high) and September (low) was greatest in youngest and this difference decreased with age. Prior to embarking on an elimination strategy, monitoring changes in transmission intensity and identification of malaria foci is mandatory for best intervention efforts. This population-based study of a malaria-endemic population identified new serological markers of infection using

genome-scale protein microarray. This new tool has important potential for providing key control and elimination data for national surveillance programs.

1511

INVESTIGATING OPERATIONAL STRATEGIES FOR ANTIMALARIAL DRUG ADMINISTRATION IN ZAMBIA'S SOUTHERN PROVINCE: A SIMULATION STUDY

Erin M. Stuckey[1], John M. Miller[2], Megan Littrell[2], Nakul Chitnis[1], Rick W. Steketee[2]

[1]Swiss Tropical and Public Health Institute, Basel, Switzerland, [2]PATH Malaria Control and Evaluation Partnership (MACEPA), Seattle, WA, United States

Malaria elimination requires reducing both the potential of mosquitoes to transmit parasites and the infectious reservoir of parasites in humans, including asymptomatic infections. To achieve this goal in Southern Province, Zambia a mass test and treat (MTAT) campaign using artemether-lumefantrine was conducted from 2011-2013 to complement high coverage of long lasting insecticide-treated nets. In order to identify factors likely to increase campaign effectiveness, a modeling approach was applied to investigate the simulated effect of alternative operational strategies for MTAT in Southern Province. OpenMalaria, a discrete-time, individual-based stochastic model of malaria, was parameterized for the range of transmission intensities observed in the study area to simulate antimalarial administration for interruption of transmission. Simulations were run for scenarios with a range of artemisinin-combination therapies (ACTs), proportion of the population reached by the campaign, targeted age groups, frequency of campaign rounds, *Plasmodium falciparum* test protocols, and the addition of drugs aimed at preventing onward transmission. Scenarios were evaluated based on the reduction in all-age parasite prevalence during the peak transmission month following the campaign, compared to the currently-implemented strategy. Simulation results suggest that the most important determinant of success in reducing prevalence is the coverage of the population achieved in the campaign. However, even with high coverage with mass drug administration (MDA) in areas with a pre-intervention all-age parasite prevalence of less than 10%, simulations suggest that elimination would require more than one year of campaign implementation. Including single low-dose primaquine, which acts as a gametocide, to the drug regimen did not further reduce prevalence. The addition of an endectocide, such as ivermectin, resulted in a lower simulated parasite prevalence and warrants further investigation. Simulation results indicate a high proportion of low density infections were missed by rapid diagnostic tests that would be treated and cleared with MDA. The optimal implementation strategy for MTAT/MDA will vary by background level of prevalence and rate of infections imported to the area. Success of the campaign depends on continued coverage of vector control interventions to ensure sustained gains in reduction of disease burden.

1512

A FREE SURVEILLANCE APP FOR PLANNING MALARIA ELIMINATION INTERVENTIONS AND OUTBREAK RESPONSES AT THE COMMUNITY LEVEL IN MALARIA ENDEMIC COUNTRIES

Antonio M. Quispe[1], Josiah L. Kephart[2], Andres G. Lescano[1], Timothy M. Shields[2], Clive J. Shiff[2]

[1]U.S. Naval Medical Research Unit - 6, Lima, Peru, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Planning malaria elimination interventions and outbreak responses at the community level represents an operational challenge in most malaria endemic countries. This happens primarily because most health systems report surveillance data at the regional and country levels only, despite data collection at the community level. Therefore, when planning interventions and responses at the community level, decision makers often need to perform their own data analysis, which is time-consuming and resource-intensive. In an attempt to address this problem, we have developed the "Free Surveillance App" (FREESAPP), an online application that converts time-series data from national surveillance systems into interactive graphs similar to those produced by the Gapminder Foundation. The applet can output burden distribution and decision-making trees for cost-effectiveness assessments as well as utilize satellite maps for background illustration. The overall goal of this application is to allow public health officers to efficiently and accurately use the surveillance data to guide their decision-making practice at each of the levels of the local health system hierarchy. To achieve this goal, FREESAPP allows users to plot the trends in malaria burden using a variety of interactive displays (i.e., bubble, bar, and line charts). FREESAP allows users to include and adjust for various covariates of interest (i.e., incident rate, population size, *P. falciparum* proportion, time, etc.) using different mathematical transformations. Users can contrast trends against epidemiological thresholds for each reporting level, which are automatically updated with each week of data entry. Furthermore, FREESAPP users are able to estimate costs of implementing interventions by using a cost-effectiveness algorithm that is adjustable by population size, distance, and coverage rates. In order to allow data managers to update the system without altering their current reporting protocol, FREESAPP was developed using a combination of four free tools: Motion Chart, Google Earth, Google maps, and R software. Given its open source free format, FREESAPP may contribute to enhancing the local readiness and response capacity at each of the levels of the Health System hierarchy in most malaria endemic countries. FREESAPP may also potentially be used for other reportable diseases, therefore facilitating improved public health decision-making.

1513

EVALUATING REACTIVE CASE DETECTION ACTIVITIES IN RANONG PROVINCE, THAILAND

Prayuth Sudathip[1], Jennifer Koonings[2], **Chris Cotter**[3], Roly Gosling[3]

[1]Bureau of Vector-Borne Diseases, Department of Disease Control, Bangkok, Thailand, [2]Global Health Sciences, University of California San Francisco, San Francisco, CA, United States, [3]Global Health Group at the University of California San Francisco, San Francisco, CA, United States

Active case detection (ACD) strategies are recommended by the World Health Organization and are implemented in many low transmission settings worldwide as a critical part of malaria elimination programs. ACD strategies include determining the origin of infection, case investigation, and responding to locally acquired cases of malaria, known as reactive case detection (RACD). Effectively implementing RACD requires substantial programmatic and human resources. Thailand is pursuing a spatially-progressive approach to eliminate malaria from 80 percent of the country by 2020. Between April and June 2014, the Thailand Bureau of Vector-Borne Diseases (BVBD) conducted an evaluation in Ranong Province to identify best practices and inform RACD efforts in the province and across Thailand. Using a standardized monitoring and evaluation (M&E) tool, five districts within the province were evaluated, and included a mix of high, medium and low transmission settings. Case investigation and RACD rates and reporting timeliness were analyzed through secondary data extraction from the national malaria information system and district-level malaria clinics and measured against defined indicators. Questionnaires were administered to 60 malaria clinic staff regarding RACD operations and procedures. A financial analysis of RACD-related expenditures was also collected and analyzed to determine the primary cost drivers and operating costs for RACD. Findings from the evaluation will inform the BVBD on program efficiency within Ranong Province, identify best practices and gaps in RACD activities, and will assist the BVBD in optimizing RACD program effectiveness.

1514

LAMP FOR THE DETECTION OF SUB-MICROSCOPIC MALARIA INFECTIONS IN THE CONTEXT OF MALARIA ELIMINATION IN CAMBODIA

Nimol Khim[1], Heidi Hopkins[2], Saorin Kim[1], Dany Dourng[1], Vincent Sluydts[3], Somony Heng[4], Lydie Canier[1], Sovanaroth Siv[4], Tho Sochantha[4], Lies Durnez[3], Marc Coosemans[3], Vincent Deubel[1], David Bell[5], Iveth J. Gonzalez[2], Didier Menard[1]

[1]Institut Pasteur in Cambodia, Phnom Penh, Cambodia, [2]Foundation for Innovative New Diagnostics, Geneva, Switzerland, [3]Institute of Tropical Medicine, Antwerp, Belgium, [4]National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia, [5]Global Good/ Intellectual Ventures Lab, Seattle, WA, United States

In the context of government's commitment to eliminate malaria in Cambodia in 2025, additional efforts and new methodologies to detect malaria parasites in asymptomatic individuals are needed. Indeed, in very low transmission settings such as Cambodia, asymptomatic infections remain the major reservoir of malaria parasites contributing to maintain disease transmission. As a consequence, the detection and treatment of the asymptomatic carriers is a crucial step in progress towards malaria elimination. This represents a new challenge as the proportion of asymptomatic parasite carriers is unknown. To date, although PCR methods show lower detection threshold compared to microscopy, Nested or Real time PCR assays required fully equipped laboratory and trained technicians. These approaches are not suitable as point-of-care in the field, contrarily to LAMP, which can be done on a simple bench top in a clinic, with basic reagents and equipment, by personnel with only a few days' training in the technique. To assess the performance of this promising tool, we have conducted a retrospective study on 516 samples from asymptomatic individuals collected in Rattanakiri province, eastern Cambodia. Ten microliters of DNA extracted by Instagene matrix from dried blood spots were used for both LAMP (Pan detection) and Real time PCR. Positive specimens with Pan LAMP were screened for falciparum species (*P. falciparum* LAMP reaction). The results between the two techniques were compared to calculate the diagnostic accuracy. Based on the LAMP detection, the prevalence of malaria infection was 19.8%. Compared to the Real time PCR, the specificity and the sensitivity of the malaria LAMP kit was 93.3% (95% CI: 90.5% - 95.4%) and 86.4% (95% CI: 76.6% - 92.7%), respectively. We concluded that LAMP detection has similar performances with the Real time PCR. In addition, LAMP results are available just one hour after sample processing begins for 14 samples. We suggest that high throughput LAMP assay for a large-scale screening would be developed for a step forward to malaria eradication.

1515

STAGE 1 TRIALS OF THE CLINICAL DEVELOPMENT PLAN FOR PFSPZ VACCINE FOR GEOGRAPHICALLY FOCUSED MALARIA ELIMINATION CAMPAIGNS

Stephen L. Hoffman

International PfSPZ Vaccine Clinical Consortium; VRC, Laboratory of Malaria Immunology and Vaccinology, DMID of National Institute of Allergy and Infectious Diseases, UMB CVD, Bamako Malaria Research and Training Center, IHI Bagamoyo, Swiss Tropical and Public Health Institute, Naval Medical Research Center, Walter Reed Army Institute of Research, University of Tubingen, MoH Equatorial Guinea, CRESIB, Centers for Disease Control and Prevention, Sanaria, Rockville, MD, United States

A vaccine for geographically focused malaria elimination campaigns must provide sustained sterile protection against infection. The Sanaria® PfSPZ Vaccine was developed to address this need. It is composed of aseptic, purified, cryopreserved, vialed PfSPZ. In a recent clinical trial at the Vaccine Research Center (VRC), NIAID, NIH, PfSPZ Vaccine protected 6/6 (100%) subjects against controlled human malaria infection at the highest dosage regimen administered (5 IV doses of 1.35x105 PfSPZ), There was a dose response in regard to antibody and T cell responses, and the vaccine was safe and well tolerated. An international consortium was established to facilitate development of PfSPZ Vaccine for use in geographically focused Pf malaria elimination campaigns, and a 4-stage clinical development plan (CDP) delineated. In Stage 1, which is in progress, trials at 3 sites in the US, and in Mali, Tanzania, Equatorial Guinea, and Germany are assessing the reproducibility of the VRC 312 trial, and optimizing durability, heterologous protection, and dosage regimens. These trials are intended to establish: (1) the reproducibility of the findings from the study conducted at VRC; (2) protection against heterologous Pf, including naturally acquired Pf; (3) durability of protection; (4) protective efficacy of different dosage regimens of PfSPZ Vaccine - regimens of 5, 4, 3, 2 or 1 doses of 1.35x105 to 2.2x106 PfSPZ/dose by direct venous inoculation (DVI) or IM routes; (5) an assay/biomarker that predicts protection; and (6) optimal approaches for DVI administration. >400 doses of 2.7x105 to 2.2x106 PfSPZ/dose have been administered in the US and Africa, and the vaccine has been safe and exceptionally well tolerated. Stage 2 will include age de-escalation and escalation and regimen optimization trials; Stage 3 will be pivotal phase 3 trials; and Stage 4 will include mass administration campaigns to halt transmission and eliminate Pf malaria from populations of > 200,000 individuals. Progress and plans will be explained.

1516

SPECTRUM OF INFECTIOUS DISEASES IN RURAL CLINIC FOR REFUGEES AND DISPLACED POPULATION ON RWANDA-DR CONGO BORDER: ANALYSIS OF 10,051 PATIENTS

Katarina Molnarova[1], Dominik Hes[1], Gertruda Mikolasova[2], Lenka Michalikova[3], Jaroslava Brnova[3], Nada Kulkova[2], **Vladimir Krcmery**[2]

[1]Bl. Clémentine-Anuarita Healthcare Centre, Bigugu, Rwanda, [2]Laboratory of Molecular Microbiology, Bratislava, Slovakia, [3]Faculty of Health Sciences and Social Work, Trnava, Slovakia

Migrants and internally displaced in sub-Saharan Africa are subject of increasing threat of infectious diseases (IDs) due to contaminated water supplies (cholera, typhoid fever), food (salmonellosis, shigellosis), malnutrition (tuberculosis-TB, HIV) and absence of housing (pneumonia, upper respiratory tract infections - RTI). Cross sectional study in area close to DRC border (Sud-Kiwu) and Rwanda (Bisesero) in two clinics serving for 50 000 population (of them 25 000 internally displaced and refugees in UNHCR camps) was performed to asses occurrence of major ID in 2013. Bigugu clinic is located in altitude of 2350 m and Bisesero United Nations High Commissioner for Refugees (UNHCR) camp is in 1150 m above sea level.Of 10 051 patients, only 31 (0,3%) had malaria, and 26 of them (0,26%) had true highland malaria (without down country travelling history), confirmed both microscopically and with rapid diagnostic test (RDT). Commonest IDs were upper RTI representing (72-89%) of all visits, followed by diarrheal and gastro-enteric diseases (13-19%). Also, 26-77% of all children were infected by geohelmints. Only one case of neuroinfection was recorded. Urinary tract infections and sexually transmitted diseases were rare as well (1-4%).Among 10 051 outpatient visits in two rural clinics, serving for UNHCR registered refugees from DRC in Rwanda and internally displaced population near Sud-Kiwu Province. Malaria was extremely rare due to high altitude, and diarrheal and gastrointestinal infections were relatively rare, too. Of all ID, upper RTI were the commonest, while neuroinfections (such as bacterial or viral meningitis and sleeping sickness) were only exceptional. Very high proportion of RTI was associated with malnutrition and very low socio-economic status in areas of high altitudes above sea level with low temperature.

1517

SPECTRUM OF INFECTION DISEASES IN BURUNDIAN RURAL HOSPITAL IN GASURA IN DRY VERSUS RAINY SEASON - 2012/2013

M. Kolibab[1], Jana Trilisinska[1], Eva Smrekova[1], Tomas Razsony[1], Eliska Maradova[1], Alexandra Mamova[1], Miriam Balazova[1], George Benca[2], Bianca Voinescu[1], **Vladimir Krcmery**[2]

[1]Health Centre in Gasura, Burundi, Gasura, Burundi, [2]Slovak Tropical Institute, Bratislava, Slovakia

Seasonal cycles of infectious diseases have been variously attributed to changes in atmospheric conditions, the prevalence or virulence of the pathogen, or the behaviour of the host. Also malaria has been observed during last 10 years with increasing frequency in areas above sea level (highlands malaria). The aim of this research was to asses if rainy/dry seasons are related with seasonal variation of infectious diseases similar to Kenya or Uganda highlands (western Kenya and south-western Uganda). Monthly incidences of malaria versus respiratory infections in June to November (rainy seasons) versus January to April (dray seasons) have been compared in 2012/2013 in community hospital in Gasura located in 1283 m.a.s. (Burundi). This hospital had about 50 beds and outpatients department. Hospital staffs was composed of 2 doctors, 8 nurses, 2 lab technicians and pharmacist, with a patient flow of 40-120 patients daily in the outpatients department and 2-10 inpatients daily. Malaria diagnosis was made microscopically (according to WHO guidelines) and was confirmed with rapid diagnostic test (RDT; according to manufacturer's instructions). Malaria was responsible for approximately 42,5 - 48,8% of all admission or consultations in rainy seasons but only 12,5% -29,7% in dry seasons. In dry season, proportion of respiratory tract infections increased from 19% in June to 42% in November and replaced malaria. Both malaria and pneumonia showed significant seasonal variations in occurrence despite of attitude of community health care centre in Gasura (1283 m.a.s.). In Burundi highlands health care centre in Gasura seasonal variations despite of high attitude (1283 m.a.s.) was observed with increasing proportion of malaria from 29,7 to 42% during rainy season replaced respiratory tract infection increased from 19 to 42% in dry season vice versa. Malaria in the Burundi highlands represented growing problem with variations in prevalence in rainy versus dry seasons.

1518

SEVERE MALARIA AMONG 3,707 ADMISSIONS IN SOUTH SUDANESE HOSPITAL FOR INTERNALLY DISPLACED POPULATION

Alexandra Mamova[1], Miriam Balazova[1], Lenka Dibusova[1], Veronika Sladeckova[1], Marian Bartkovjak[1], Juraj Benca[1], John Mutuku Muli[1], Lenka Michalikova[2], Nada Kulkova[3], Jaroslava Brnova[2], Gertruda Mikolasova[3], Maria Raucinova[1], Jirina Kafkova[1], **Vladimir Krcmery**[4]

[1]St. Francesis of D´Assisi Mission Hospital, St. Elizabeth Tropical Program Marial Lou, Mapuordit, South Sudan, [2]Trnava University, Faculty of Health Sciences and Social Work, Department of Theoretical Disciplines and Laboratory Investigation Methods, Trnava, Slovakia, [3]Laboratory of Molecular Microbiology, St. Elisabeth University College of Health and Social Science, Bratislava, Slovakia, [4]St. Elisabeth University College of Health and Social Science, Bratislava, Slovakia

Severe malaria is responsible for 1.2 million deaths worldwide, 90% of them in Sub-Saharan Africa, mainly in children below 5 years of age. The aim of this study was to assess proportion of severe malaria among all admissions hospital in area with internally displaced population in south Sudan. Data on infectious diseases were analyzed at admission within 12 months 1.1. 2013 - 31.12.2013 at St. Francesis of D´Assisi mission Hospital located in Marial Lou, South Sudan, built for internally displaced refugees coming from north to South Sudan due to civil war (1982 - 2005) and Darfour Conflict (2002 - 2012)and for about 50 000 Dinka population. Diagnosis of severe malaria was done clinically, plus microscopically, plus rapid diagnostic testing (RDT) has been used since 2013. Seasonality of severe malaria was observed among majority of cases in period from April to November, with 113 to 221 cases per month with up to 9 deaths on severe malaria (monthly among 3707 admissions, in 2013). Altogether, 1438 patients (38.8 %) had severe malaria clinically confirmed as fever plus severe anemia, or respiratory distress syndrome, or cerebral malaria, or liver, or kidney failure, or severe hypoglycemia with acidosis. Of 1438 severe malaria cases, 76 died (5.3 %). Relatively low mortality may be explained with: (i) good access to the hospital, (ii) pre-referral administration of antimalarial drugs due to education campaign with the in 2010 - 2013, and (iii) use of artemisinin-based combination therapy (ACT) since 2010 in Marial Lou. Severe malaria in travelers returning to Europe is associated with up to 20 % mortality. But it can by moresuccessfully treated on site in tropics due to semi-immune population, early pre-referral administration of antimalarial drugs and early empiric intramuscular and venous administration of antimalarial drugs; resulting to 5 % mortality even in more severe cases.

1519

GENETIC DIVERSITY AND POPULATION STRUCTURE OF *PLASMODIUM VIVAX* INFECTIONS AFTER RADICAL TREATMENT IN A RURAL COMMUNITY OF CENTRAL VIETNAM

Nguyen Van Hong[1], Christopher Delgado-Ratto[2], Pham Vinh Thanh[1], Peter Van Den Eede[3], Nguyen Thi Huong Binh[1], Umberto D'Alessandro[3], Tran Thanh Duong[1], Jean – Pierre Van Geertruyden[2], Annette Erhart[3], Anna Rosanas-Urgell[3]

[1]National Institute of Malariology, Parasitology and Entomology, Hanoi, Vietnam, [2]Faculty of Medicine, University of Antwerp, Antwerp, Belgium, [3]Institute of Tropical Medicine, Antwerp, Belgium

In Vietnam the malaria burden has been drastically reduced over the past two decades but *Plasmodium vivax* is becoming increasingly important mainly due to its relapsing nature and the difficulty to radically cure dormant parasites from the liver. A two-year cohort study was conducted in Central Vietnam to assess the efficacy of the radical cure regimen based on a 10-day Primaquine (0.5mg/kg/d) in combination with the standard 3-day chloroquine (total 25mg/kg) regimen. We report the genetic diversity and population structure of *P. vivax* infections before and after radical treatment. All day 0 (n=247) and post treatment *P. vivax* infections (n=788) detected by microscopy and PCR during the 2-year monthly follow-up were genotyped using 16 previously described microsatellites. Genetic diversity, linkage disequilibrium, population structure and haplotype clustering were analyzed in post treatment samples and compared to Day 0 samples. All markers were highly polymorphic with 3 to 30 alleles per marker and heterozigosity (He) values ranging from 0.35 to 0.90. Overall He values were not significantly different between day 0 and post-treatment samples (He = 0.64 and 0.66 respectively). In addition, 71.0% of all infections were polyclonal (76.9% at day 0 vs. 69.2% post-treatment samples) and the average multiplicity of infection (MOI) was 1.9 parasites/person (MOI = 2.1 at D0, MOI =1.8 at recurrences). Genetic diversity of parasite population experimented significant changes when parasite population before treatment was compared with parasite population in the second year follow up (FST= 0.21). which may suggest a delayed effect of the intervention or may reflect the intense follow up (with treatment of all cases) study design. In order to estimate multilocus linkage disequilibrium (LD) changes between day 0 and post-treatment samples, we calculated the index of association IsA (which is zero for LD). We observed higher LD in post-treatment than day 0 parasite population (IsA = 0.093, P=0.0001 and IsA = 0.039, P=0.0001, respectively), suggesting inbreeding and a clonal population structure. Overall parasite population in the study is genetically diverse, and has a low effective recombination rate that contrasts with the high number of polyclonal infections.

1520

SEASONALITY IN MALARIA TRANSMISSION - IMPLICATIONS FOR CASE-MANAGEMENT WITH LONG-ACTING ARTEMISININ COMBINATION THERAPIES

Matt Cairns[1], Patrick Walker[2], Lucy Okell[2], Jamie Griffin[2], Tini Garske[2], Kwaku Poku Asante[3], Seth Owusu-Agyei[3], Diadier Diallo[4], Alassane Dicko[5], Badara Cisse[6], Brian Greenwood[1], Daniel Chandramohan[1], Azra Ghani[2], Paul Milligan[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]MRC Centre for Outbreak Analysis and Modelling, Imperial College London, London, United Kingdom, [3]Kintampo Health Research Centre, Kintampo, Ghana, [4]London School of Hygiene & Tropical Medicine, now at PATH-Malaria Vaccine Initiative, Dakar, Senegal, [5]Malaria Research and Training Center, Bamako, Mali, [6]Universite Cheikh Anta Diop, Dakar, Senegal

Seasonality in malaria transmission is a key driver of malaria epidemiology, and has important implications for the effectiveness of interventions. One aspect that is not well understood is how seasonality affects the number of repeat malaria episodes that occur soon after a previous clinical attack, and which might be prevented if a long-acting artemisinin combination therapy (LACT) was used to manage the initial episode. We estimated the separate and combined effects of transmission intensity and seasonality on the timing and concentration of repeat malaria episodes, using data from six cohort studies in West Africa, and then used an individual-based model of malaria transmission across sub-Saharan Africa to extrapolate these results across a range of settings. Seasonality was quantified using the Markham seasonality index (MSI), taking account of areas with bimodal seasonality patterns, and the concentration of malaria episodes in time was quantified using a modified version of the Gini index. We explored 10% intervals of the MSI, and simulated transmission intensity that equates to prevalence in 2-10 year olds ranging from 5-60%. In settings where prevalence is less than 10%, repeat malaria episodes constitute a small fraction of the total burden, and few repeat episodes occur within the window of protection provided by currently available drugs. However, in higher transmission settings, and particularly in highly seasonal settings, repeat malaria becomes increasingly important, with up to 20% of the total clinical burden in children estimated to be due to repeat episodes within four weeks of a prior attack. At a given level of transmission intensity and annual incidence, the concentration of repeat malaria episodes in time, and consequently the protection from LACTs, is always highest in the most seasonal areas. As a result, the degree of seasonality, in addition to the overall intensity of transmission, should be considered by policy makers when deciding between ACTs that differ in terms of the duration of post-treatment prophylaxis they provide.

1521

DETECTING FOCI OF MALARIA TRANSMISSION: IMPLICATIONS OF SAMPLE SIZE AND CHOICE OF MALARIA METRIC

Gillian H. Stresman[1], Emanuele Giorgi[2], Amrish Baidjoe[3], Philip Knight[4], Wycliffe Odongo[5], Chrispin Owaga[5], Shehu Shagari[5], Euniah Makori[5], Jennifer Stevenson[6], Chris Drakeley[1], Jonathan Cox[1], Peter Diggle[2], Teun Bousema[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Lancaster University, Lancaster, United Kingdom, [3]Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands, [4]University of the West of England, Bristol, United Kingdom, [5]KEMRI/Centers for Disease Control and Prevention, Kisumu, Kenya, [6]Johns Hopkins Bloomberg School of Public Health, Macha, Zambia

Identifying malaria foci in endemic settings can be challenging. Many pragmatic decisions must be made, including the sample size to employ and which malaria metric to use. However, it is not known how these decisions affect the ability to detect foci of infection, particularly the boundaries of foci identified. If control or elimination programs are targeted to foci of infection that do not accurately reflect the true nature of transmission in the community, ensuing interventions may incorrectly be perceived as being ineffective. To determine the impact of sample size and choice of malaria metric, data for 17,500 individuals residing in 3,200 compounds, approximately one third of the population, collected during a cross-sectional survey in the western Kenyan highlands were used to identify foci of transmission in the community. All structures in the area were digitized to provide a total census of the area and several structures can comprise a compound. Model-based geostatistical methods were used to analyze the spatial variation of parasite prevalence, as determined by polymerase chain reaction (PCR), a measure for current infection, and by seropositivity, a measure of malaria exposure. Informative thresholds of risk were defined in order to identify foci in the spatial distribution of the two outcomes. The impact of the sample size on both the accuracy of prevalence estimates and the ability of the model to identify foci was assessed through a simulation study. Preliminary findings suggest that foci defined by the two outcome measures were measures were only moderately correlated (r=0.43) with only 36% of structures identified by both outcome measures. Among 14 discrete foci identified as having increased risk by one or both outcomes 6 clusters were identified by both metrics, although only 3 had good overlap. Five clusters (592 structures) were identified based on PCR but missed using seroprevalence, and 3 clusters (1214 structures) were missed using PCR but identified using seroprevalence. In terms of sample size, initial findings indicate that halving the sample size would have a minimal impact on model efficiency for generating the predicted surface for both outcome measures.

1522

UTILITY OF PCR-BASED SURVEILLANCE METHODS RELATIVE TO AGE IN RAINY AND DRY SEASONS IN SOUTHERN MALAWI

Jenny A. Walldorf[1], Jenna E. Coalson[2], Andy Bauleni[3], Kondwani Nkanaunena[3], Clarissa Valim[4], Lauren M. Cohee[1], Nelson Chimbiya[3], Jacqueline Fiore[5], Atupele Kapito-Tembo[3], Karl Seydel[6], Don Mathanga[3], Terrie E. Taylor[6], Miriam K. Laufer[1]

[1]University of Maryland Baltimore, Baltimore, MD, United States, [2]University of Michigan School of Public Health, Ann Arbor, MI, United States, [3]Malaria Alert Center, Blantyre, Malawi, [4]Harvard School of Public Health, Boston, MA, United States, [5]College of Osteopathic Medicine, East Lansing, MI, United States, [6]Blantyre Malaria Project, Blantyre, Malawi

Routine malaria surveillance often relies on microscopic or rapid diagnostic test (RDT) parasite detection, which misses low density infections. We have previously shown in cross-sectional surveys that school age children are at high risk of PCR-detectable asymptomatic infections in dry and rainy seasons. Using the same survey data, we now assess risk factors for low density infection to determine the utility of PCR by age and transmission setting. Submicroscopic infection was defined as *Plasmodium falciparum* detected by PCR but with a negative malaria smear read by quality-controlled microscopy. Sub-RDT infection was defined as PCR detection with microscopy negative or <200 parasites per microliter. Among all PCR-detected infections, infections were more likely to be submicroscopic in the dry (56%,180/319) vs. rainy season (38%, 208/544, p<.0001). The proportion of infections that were submicroscopic among adults (≥16 years), school age (6-15 years), and young children (≤5 years), was 72% (69/96), 55% (93/168), and 33% (18/54) in the dry season and 56% (99/178), 30% (81/274), and 30% (27/91) in the rainy season, respectively (p<.0001 both seasons). In mixed modeling, relationships between age and low density infections varied by season. In the rainy season, adults had 3.0 [95% CI: 1.7, 5.2] increased odds of submicroscopic infection, while school age children did not differ from young children. In the same communities in the dry season, both adults (OR 5.3 [2.6, 11.0]) and school age children (OR 2.5 [1.3, 5.0]) had increased odds of submicroscopic infection relative to young children. Associations were independent of district, net use, house materials, and gender. The relationship between age and sub-RDT infections followed a similar pattern. Microscopy and RDTs have inconsistent performance across age groups and seasons. While

both microscopy and molecular detection have demonstrated the highest malaria burden among school age children, surveillance with microscopy or RDT alone fails to detect most infections among adults and half of the infections in school age children.

1523

ESTIMATING THE MALARIA ATTACK RATE OF A TANZANIAN MILITARY COHORT IN THE SEARCH FOR NON-IMMUNE POPULATIONS FOR MALARIA PROPHYLAXIS, VACCINE AND TREATMENT STUDIES

Charles E. Mwanziva[1], Shekalaghe Seif[2], Lucky Temu[3], Sarah Chiduo[4], George Amoo[5], David Schnabel[6], Lalaine Anova[6], Colin Ohrt[6], Eyako Wurapa[6], Akili Kalinga[7], Christopher Mswanya[1], Yadon M. Kohi[1], Adam S. Mwambulanga[1]

[1]Tanzania People's Defence Forces, Dar es Salaam, United Republic of Tanzania, [2]Ifakara Health Institute, Bagamoyo, United Republic of Tanzania, [3]Tanzania People's Defence Forces, Walter Reed Program - Tanzania, United Republic of Tanzania, [4]Walter Reed Program - Tanzania, Dar es Salaam, United Republic of Tanzania, [5]Amethyst Technologies LLC, Baltimore, MD, United States, [6]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [7]National Institute for Medical Research, Tukuyu, United Republic of Tanzania

In Tanzania, malaria is the leading cause of outpatient and inpatient health service attendance, and accounts for about 32% of hospital deaths. The disease is unevenly distributed in the country. Some areas of Tanzania have little to no malaria transmission as a result of anti-malarial interventions (e.g., use of treated bednets and ACT) as well as differences in climate and geography, while other areas are highly endemic for malaria. Exposure to the malaria parasite gives individuals the ability to develop immunity and asymptomatic infection. However, naive populations from low-risk areas have no protective immunity and are at high risk of developing the disease. This study aims at determining the malaria attack rate of proposed non-immune individuals from non-malarious areas when entering a training camp in a highly endemic area. 500 recruits from Tanzania People's Defence Forces (TPDF) from non-endemic areas were selected by multistage random sampling; consenting, eligible participants were followed for six months. Malaria smears were collected every fortnight by active and passive detection of infection at the camp health facility. Blood samples for PCR and serological tests were collected. Malaria diagnosis was confirmed by malaria microscopy. There was a high rate of study subject follow-up; 98.1% (491/500) individuals participated in all activities, while 8 withdrew their participation for personal reasons. 21% (102/491) were terminated after confirmed malaria infection by clinical laboratory test (study end point), and one participant died from a non-malarial infection. The malaria attack rate was found to be as low as 18% to above 24%. *Plasmodium falciparum* was the predominant species detected. This study revealed one of very few non-immune populations with sufficient malaria exposure to conduct malaria prevention studies of a reasonable size, made possible by the heterogenous disease distribution in the country. TPDF recruits from non-endemic areas may be an ideal non-immune population for future malaria prophylaxis, vaccine and treatment trials.

1524

MALARIA INCIDENCE IN A DISTRICT WITH THREE ECOLOGICAL ZONES IN SOUTHERN GHANA

Alberta A. Quartey

Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Information on malaria incidence,age patterns of morbidity,mortality and serological exposure can be combined with entomological data to help target enhanced malaria control. Although the Dangme West District in Ghana hosts a research centre and has conducted many malaria interventions,the last malaria transmission measurement was undertaken in1994.We have therefore undertaken a new detailed malaria transmission study stratified by ecological zone. We followed a cohort of 2145 participants of all ages(715 per zone, selected by multistage cluster sampling)once a month from April 2011 to March 2012.A history of fever within the previous 2weeks was elicited at each visit and further information obtained by questionnaire from those with a positive history, after which a finger-prick blood sample was taken for the preparation of blood smears. Data was analyzed in STATA12.We completed 77% of all planned visits;8% of participants reported fever,3% had used an artemisinin combination therapy (ACT) for treatment of perceived fever and 6% had used an insecticide treated bed-net (ITN)the night before visits.The incidence of slide confirmed malaria per 1000 person years was 85 in the Forest,41 in the Coastal and 13 in the Lakeside zones.Verified ITN use the night before visits in each of the zones was 3%,4% and 9% respectively.The absence of a ceiling in a room was associated with an excess risk of malaria of 15%. Malaria incidence per 1000 person years was 119 in those aged 0-4years,136 in those aged 5-9,50 in those aged 10-19, 9 in those aged 20-29,18 in those aged 30-39 and 24 in those over 40 years of age.Overall rates had decreased by 40% from the 1994 levels.The Lakeside zone had the lowest incidence despite vast irrigated fields and the lowest access to ACTs.The Forest zone with the lowest verified ITN use and ownership(25%) and highest access to ACTs bore the brunt of morbidity.The data suggest that in an area of declining malaria transmission,efficient surveillance is required to promptly determine levels and patterns of morbidity to identify remaining foci for targeted interventions

1525

CONTRIBUTIONS OF WOMEN WITH CHILDREN AND YOUTH WORKERS TO SPATIAL MALARIA TRANSMISSION IN SUB-SAHARAN AFRICA

John M. Marshall[1], Mahamoudou B. Touré[2], André Lin Ouédraogo[3], Micky Ndhlovu[4], Samson S. Kiware[5], Ashley Rezai[1], Emmy Nkhama[4], Jamie T. Griffin[1], T. Deirdre Hollingsworth[6], Seydou Doumbia[2], Nico Govella[5], Neil M. Ferguson[1], Azra C. Ghani[1]

[1]Imperial College London, London, United Kingdom, [2]Malaria Research and Training Center, Bamako, Mali, [3]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso, [4]Chainama College of Health Sciences, Lusaka, Zambia, [5]Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania, [6]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Recent investment in global malaria control has led to malaria prevalence dropping in many parts of the world. As people play a dominant role in parasite dispersal, a quantitative understanding of human movement patterns is relevant to determining how best to maintain gains made through these efforts. We conducted a survey of human movement patterns in four countries throughout sub-Saharan Africa - Mali, Burkina Faso, Zambia and Tanzania - with additional questions on malaria risk factors and cell phone usage behavior, the latter to enable anonymous cell phone signal data to be better-correlated with movement patterns. A total of 4,352 individuals were interviewed and 6,141 trips recorded. A cluster analysis highlighted two distinct traveler groups of relevance to malaria transmission - women traveling with children (all four countries) and youth workers (Mali). Women with children were predominantly between the ages of 16 and 45 and were more likely to travel to areas of relatively high malaria prevalence in Mali ($p<0.001$) and Zambia ($p=0.035$) compared to other travelers. They were also more likely to own bed nets in Burkina Faso ($p=0.001$) and Zambia ($p<0.001$), to use bed nets in Zambia ($p<0.001$) and Tanzania ($p=0.046$), and to own a cell phone in Mali ($p<0.001$), Burkina Faso ($p<0.001$) and Zambia ($p<0.001$). Taking into account that children are especially receptive to malaria parasites, women with children were estimated to account for the majority of spatial malaria transmission in Mali, Burkina Faso and Zambia. Malian youth workers were predominantly between the ages of 16 and 29 and were more likely to travel to areas of relatively high malaria prevalence ($p<0.001$) and for longer durations ($p<0.001$) compared to other travelers. They were estimated to make a

significant contribution to spatial malaria transmission in Mali. Knowledge of the spatial patterns of malaria transmission and the contributions of key traveler groups to this spread will assist in the design of control and surveillance programs targeting "hot spots" of malaria transmission.

1526

EPIDEMIOLOGY OF MALARIA INFECTION AMONG SCHOOL-AGED CHILDREN IN KINTAMPO NORTH DISTRICT, GHANA: AN EVALUATION OF BEHAVIOR, NUTRITIONAL STATUS, HOOKWORM CO-INFECTION AND ANTIBODY RESPONSES

Jennifer Du Mond[1], Benjamin T. Simms[1], Dylan Davey[2], Joseph Otchere[3], Debbie Humphries[1], Josephine Quagraine[3], Samuel Newton[4], Lisa Harrison[2], Daniel Boakye[3], Michael Wilson[2], Michael Cappello[2]

[1]Yale University School of Public Health, New Haven, CT, United States, [2]Yale University School of Medicine, New Haven, CT, United States, [3]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [4]Kintampo Health Research Center, Brong Ahafo, Ghana

A cross-sectional study was conducted in June 2010 in the Kintampo North Municipality, Ghana. Children (n=286) from 16 schools were enrolled after screening (n=844) if they had HAZ Z-score below -1.80 or above -0.10, with no more than one child from each household. Serum, fecal samples, and household surveys were used to assess the associations between the presence of malaria parasites, malaria parasitemia, and individual and household risk factors including nutritional status, hookworm co-infection, household risk prevention behaviors, and serum measures of parasite-specific immunoglobulin G (IgG). The primary factors associated with reduced risk of malaria infection included spraying the house in the past year (OR=0.04, p<0.001), the child having a health care visit in the past year (OR=0.39, p<0.001), household malaria in the past year (OR=0.37, p=0.001), higher hookworm antibody levels, and geographic location, while greater household food insecurity was associated with reduced risk of high levels of parasitemia. Primary risk factors for elevated parasite density included the house being sprayed in the past year (OR=9.83, p<0.001), household bednet usage (higher proportion of use associated with greater parasitemia), household and child history of malaria in the past year (OR=2.80, p=0.039; OR=0.15, p<0.001, respectively), frequency of consumption of protein-rich food groups, and geographic location, while those with the highest hookworm antibody levels showed reduced parasite density. Hookworm infection was associated with increased risk of malaria infection (OR=2.65, p=.10) and higher density of malaria parasites among those infected (OR=2.81, p=0.001). These risk factors highlight areas of programmatic interest, particularly the elevated risks of malaria infection and higher density of parasites among those infected with hookworm. Further research should elucidate the mechanism of this interaction, and treatment measures should focus on reducing the burden of hookworm in malaria endemic areas.

1527

PREVALENCE OF TWO NEWLY RECOGNIZED HUMAN MALARIA SPECIES IN MALI: *PLASMODIUM OVALE CURTISI AND P. OVALE WALLIKERI*

Nouhoum Diallo[1], Sangare P. Cheick[1], Antoine Dara[1], Aboubacrine Haidara[1], Bakari Fofana[1], Bakari Sidibe[1], Souleymane Dama[1], Omar B. Traore[1], Aliou Traore[1], Sekou Toure[1], Amadou Togo[1], Niawalou Dara[1], Moctar Coulibaly[1], Issaka Sagara[1], Khalid Bechir[2], Colin Sutherland[2], Ogobara K. Doumbo[1], Abdoulaye Djimde[1]

[1]University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom

Ovale malaria is caused by two sympatric *Plasmodium ovale* sub-species; their biology and morbidity need to be investigated. In the present study we determine the presence and the prevalence of these sub-species in Mali using molecular analysis of blood blotted onto filter papers. Between 2011 and 2013, 7044 volunteers were screened by light microscopy in the context of various clinical studies conducted in five sites of Mali with different malaria epidemiology (Bougoula-Hameau, Faladje, Kolle, Pongonon and Sotuba). Thick smears were made and read onsite by experienced microscopists. Genomic DNA was extracted using Qiagen kits. First, ssrRNA-based PCR methods detecting the *P. ovale* specy were performed. Second, nested PCR of *P. ovale tryptophan-rich antigen (potra)* gene designed to distinguish the sub-species *P. ovale. curtisi and P. ovale. wallikeri* were run. Overall, 84/7044 (1.2%) of slides were positive for P. ovale. To date, 483 dried blood spots were analyzed by PCR. ssrRNA analysis revealed 12 (2.5%) cases of *P. ovale*. Potra analysis showed that 6/12 (50%) were *P. ovale curtisi*, 5/12 (41.7%) were *P. ovale wallikeri* and 1 sample was not sub-typable. We show the two recently described *P. ovale* sub-species were both present in Mali.

1528

USING DHIS2 FOR ROUTINE MONITORING OF QUALITY OF HEALTH SERVICES IN THE PRIVATE SECTOR

Purity Njagi[1], Martin Dale[1], Victor Lara[1], Preston Izulla[2], Stephanie Dolan[1], Nikki Charman[1]

[1]Population Services International, Nairobi, Kenya, [2]PS Kenya, Nairobi, Kenya

District Health Information System (DHIS2) has increasingly become the preferred Health information system for effective management of health data in many countries, mostly for collection and management of routine service data at various levels of the health sector. However, DHIS2 capabilities can also be optimised for routine monitoring of quality of health services in the private sector: Population Services International (PSI) is implementing a multi-country project aiming to create a market for malaria Rapid Diagnostic Tests (mRDTs) to improve quality of care in the private health sector by enabling effective treatment based on diagnosis. This project is being implemented by PSI in Kenya, Madagascar and Tanzania. The use of DHIS2 for the purpose of tracking provider level case management will be presented, with specific focus the power of DHIS2 to convert routine data into decision-making. DHIS2-enabled tablets can be used to (i) undertake provider quality of care and service preparedness assessments, (ii) automatically score and benchmark the provider's performance on site, and (iii) provide effective on the spot feedback for continuous improvement. The power of DHIS2 dashboards to manage and give feedback to providers will be highlighted, bridging information on provider quality of service, productivity based on caseloads, and behaviour change based on the adoption stairway. In addition, an innovative adaptation of DHIS2 to allow effective allocation of resources through automated supervision planning, taking into account provider quality of service benchmarks will be discussed (frequency and scheduling of provider assessments in particular). The presentation will further demonstrate how DHIS2 can enable program managers to effectively track service provision and make informed decisions leading to program quality improvement through the use of tailored dashboards. Potential future links with national systems will also be discussed, given the widespread use of DHIS2 by governments in the countries we work.

1529

USE OF SATELLITE IMAGERY TO ESTABLISH A SAMPLING FRAME AND MEASURE HOUSEHOLD MOVEMENT IN SOUTHERN ZAMBIA BETWEEN 2007 AND 2011

Timothy M. Shields[1], Jessie Pinchoff[1], William Moss[1], Jailos Lubinda[2], Harry Hamapumbu[2], Frank Curriero[1]

[1]Johns Hopkins School of Public Health, Baltimore, MD, United States, [2]Johns Hopkins School of Public Health, Macha, Zambia

High-resolution satellite imagery can be used to establish a sampling frame for epidemiologic research and to describe patterns of household

distribution and movement. Assessing the frequency and geographic distribution of household movement by comparing satellite images taken over time may suggest a time period for satellite image accuracy and utility for epidemiological research. All households in a 575 km2 region of southern Zambia were enumerated based on satellite images taken in 2007 and in 2011. Movement of households in the study area was assessed by comparing the images to calculate the percentage of households that were built, removed or stayed the same. We created a spatial intensity map to identify geographic areas of household movement, and to describe the spatial variation in household movement. There were a total of 3,287 household enumerated in 2007 and 3,721 in 2011. 970 households were newly observed in 2011 and 536 were no longer present. Reporting a net change of 434 households occurring over the four year period does not adequately describe the population movement within this region. Spatial variation around key features, such as around the new sealed road, points to non-uniform dynamics in population movement. These population dynamics may have implications for field studies working in this area over this time period.

1530

REDEFINING THE URBAN-RURAL CONTINUUM FOR MALARIA RISK: NEW APPROACHES TO CHARACTERIZING PATTERNS IN MALAWI

Peter S. Larson[1], Joseph N. Eisenberg[1], Carl H. Campbell[2], Don P. Mathanga[3], Mark L. Wilson[1]

[1]University of Michigan School of Public Health, Ann Arbor, MI, United States, [2]University of Georgia, Athens, GA, United States, [3]University of Malawi College of Medicine, Blantyre, Malawi

Urban and rural setting is often included in the analysis of infectious disease risk, as it may be an important determinant of pathogen transmission. This dichotomous designation, however, is politically defined, and masks the true biological and social causal determinants of risk. To improve understanding of disease patterns and targeting of interventions, transmission-specific characterization of the urban-rural continuum is needed. We developed and tested a composite measure for malaria in Malawi that included features such as health facilities, roads, rivers, lakes and electricity, along with census-derived population density. Analysis was based on a household-level survey of infection and various risk factors among 6-59 month old children. Geographic distances to features were estimated and a principal component analysis (PCA)-based composite measure for each household was produced for all points on a fine-scale grid throughout Malawi. Statistical relationships of all factors were tested against *Plasmodium* parasitemia status using multivariate regression based methods, including potential household-level confounding factors such as treated net use and material wealth. Urban-like and rural-like areas existed throughout Malawi, even within areas classified as "urban" and "rural" by the Malawi Government. Individual factors associated with urban and rural divides, including proximity to health services and roads, as well as population density, were associated with *Plasmodium* infection. Community-level factors associated with human settlements and urban development were predictive of decreased malaria risk, even in the presence of more traditional household-level prevention methods such as ITN use. Infection probability was similar for most "rural" areas, but declined linearly after a breakpoint with increasing urbanicity, as measured by the PCA based composite. Dichotomized measures of urban and rural spaces fail to adequately characterize environments which might be associated with risks for infectious disease transmission. Politically determined destinations may ignore "urbanized" pockets within traditionally "rural" areas, and rural-like spaces within areas classified as "urban." A composite measure which analyzes many factors associated to varying degrees with spaces roughly defined as rural and urban presents an opportunity to refine places of disease risk.

1531

MALARIA ATTRIBUTABLE FRACTION TO FEVER IN A COHORT OF PAPUA NEW GUINEAN CHILDREN

Anna Rosanas-Urgell[1], Inoni Betuela[2], Benson Kiniboro[2], Danielle I. Stanisic[2], Lorna Samol[2], Leanne J. Robinson[2], Peter Siba[2], Ivo Mueller[3]

[1]Institute of Tropical Medicine, Antwerp, Belgium, [2]Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea, [3]Walter and Eliza Hall Institute, Parkville, Australia

In areas of high malaria endemicity, a high proportion of individuals is asymptomatic, thus are infected with malaria parasites without developing clinical symptoms. In addition, those who develop symptoms in the presence of malaria parasites, may be ill due to non-malaria diseases. Therefore, a case definition able to distinguish between malaria and non-malaria morbidity is essential to correctly estimate the burden of disease, design adequate control strategies -including case management-, and measure the effect of malaria interventions. In a cohort of 500 Papua New Guinean children aged 1 to 5 years and followed up for 9 months, logistic regression methods were used to estimate the risk of fever by parasite density above specific cut-offs. *Plasmodium falciparum* and *P. vivax* attributable fraction (AF) and population attributable fraction (PAF) to fever were calculated from odds ratio estimates for each case definition. Sensitivity and specificity of case definitions were also evaluated. *P. falciparum* AF ranges from 79% to 94% when all parasite densities and densities higher than 50000 P/µL are used as cut-off values, respectively. Overall, the PAF of fever to *P. falciparum* was 16% when all parasite densities in the presence of fever were considered. On the other hand, *P. vivax* AF increased from 9% (all parasite densities) to 85% when parasites densities higher than 10000 P/µL were used. *P. vivax* PAF exhibit the highest values (5-7%) when a cut-off of >1500 P/µL was used. Estimates of the sensitivity and specificity of case definitions cut-off by parasite density show that a low *P. falciparum* cut-off (<2500 P/µL) achieves high sensitivity (80-100%), while when only using high parasite density cut-off values high specificity (90%) is obtained. 80% sensitivity and specificity is achieved with *P. vivax* cut-off value > 10000 P/µL.Approximately 80% to 94% of fevers with *P. falciparum* infections occurring in Papua New Guinean children aged 1 to 5 years are attributable to malaria regardless of parasite densities. On the other hand, only 9% of fevers occurring in the presence of *P. vivax* infections, at any parasite density, are attributable to malaria, suggesting that clinical tolerance against low density *P. vivax* infections is already acquired at this young age.

1532

PRIVATE SECTOR READINESS FOR MALARIA CASE MANAGEMENT AND MALARIA MARKET COMPOSITION BEFORE AND AFTER THE AFFORDABLE MEDICINES FACILITY - MALARIA (AMFM): RESULTS FROM THREE PILOT COUNTRIES

Megan Littrell[1], Julius Njogu[2], Raymond Sudoi[2], Kevin Duff[2], Hana Bilak[3], Vamsi Vasireddy[4], Kate O'Connell[5], Stephen Poyer[2]

[1]Population Services International, Washington, DC, United States, [2]Population Services International, Nairobi, Kenya, [3]Population Services International, Chevry, France, [4]Independent Consultant, Chicago, IL, United States, [5]Independent Consultant, Yangon, Myanmar

People living in malaria endemic countries often turn to the private sector for fever case management. However, private sector markets are typically characterized by low levels of readiness for appropriate malaria case management, particularly in comparison with public health facilities. Efforts to improve this have included the Affordable Medicines Facility - malaria (AMFm), which aimed to improve availability and affordability of quality assured Artemisinin Combination Therapy (ACT) in both public and private sectors. The pilot demonstrated favorable improvements in private sector readiness for malaria case management in most countries. The private sector comprises a diverse set of actors, including regulated pharmacies and health facilities, and unregulated drug shops and general

retailers. We examine the level of private sector readiness post-AFMm, and compare private sector market composition before, during, and after the pilot in Madagascar, Nigeria and Uganda. Trends in key readiness indicators are examined, including availability of blood testing and ACT, and provider knowledge. Favorable trends in private sector readiness may be driven by improvements across existing market actors, or may be an indirect effect of shifting market composition towards a market dominated by regulated market actors. In the context of varying degrees of improvement, we examine the private sector malaria market composition over time. Multiple nationally representative outlet surveys were conducted between 2009 and 2013 by the ACTwatch project in the 3 countries. During each survey, a census of all outlets with the potential to sell antimalarials was conducted, allowing examination of relative market composition and antimalarial market share between and within sectors. Private sector readiness improved over time in all 3 countries although to varying degrees across contexts and measures of readiness. We examine readiness trends over time in relation to shifts in market composition, and discuss implications for improving private sector readiness for malaria case management.

1533

TRENDS IN AVAILABILITY OF MALARIA MEDICINES AND DIAGNOSTICS IN KINSHASA, DR CONGO FROM 2009-2013

Julie Archer[1], Godéfroid Mpanya[2], Stephen Poyer[1], Antoinette Tshefu[3], Willy Onema[2], Jamie Ciesla[2], Joris Losimba Likwela[4], Vamsi Vasireddy[5], Megan Littrell[6]

[1]Population Services International, Nairobi, Kenya, [2]Association de Santé Familiale/PSI, Kinshasa, Democratic Republic of the Congo, [3]Ecole de Santé Public de Kinshasa, Kinshasa, Democratic Republic of the Congo, [4]National Malarial Control Program, Kinshasa, Democratic Republic of the Congo, [5]Independent Consultant, Chicago, IL, United States, [6]Population Services International, Washington, DC, United States

Malaria is a leading cause of illness and death in the Democratic Republic of the Congo (DRC) where the malaria disease burden is estimated to be the second highest in the world. Malaria is endemic throughout the country, including in the large urban agglomeration of Kinshasa. National policy has recommended diagnostic testing since 2006, and the national treatment protocol includes two artemisinin-based combination therapies (ACT) for first-line treatment. However, according to past surveys, access to and use of malaria diagnostics and ACT remains low. A 2009 survey conducted by ACTwatch found that 60% of antimalarial-stocking public and private sector outlets in Kinshasa had ACT in stock, but a 2010 ACTwatch household survey found that just 15% of children with fever had received a diagnostic test for malaria and only 4.5% of those with fever had received an ACT. In a late 2013 follow-up to the 2009 survey, ACTwatch conducted a representative cross-sectional survey of 3,654 public and private sector health facilities and retail outlets in Kinshasa to assess the availability, price and market share of antimalarials and malaria diagnostics. Findings from this survey and a trend analysis will be presented. Preliminary results show that availability of malaria diagnostics remains low and the antimalarial market is still dominated by quinine in the private sector. Results will be examined in the context of qualitative research findings from in-depth interviews conducted in 2014 in Kinshasa with private doctors, pharmacists, drug shop owners and clients. There are renewed efforts in DRC to increase the quality of fever case management following World Health Organization recommendations calling for universal parasitological confirmation before treatment. This survey of the landscape of availability of ACT and malaria diagnostics, as well as ACT market share will inform renewed case management efforts in Kinshasa that can then be scaled up throughout the country.

1534

EFFECTS OF AGE AND CONTROL INTERVENTIONS ON PREVALENCE OF *PLASMODIUM FALCIPARUM* GAMETOCYTEMIA DURING PEAK MALARIA TRANSMISSION SEASON IN WESTERN KENYA

Zhiyong Zhou[1], Rebecca M. Mitchell[1], Simon Kariuki[2], Christopher Odero[2], Peter Otieno[2], Kephas Otieno[2], Vincent Were[2], Mary Hamel[1], John Gimnig[1], Edward D. Walker[3], Meghna Desai[1], Ya Ping Shi[1]

[1]Malaria Branch, Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Kenya Medical Research Institute, Kisumu, Kenya, [3]Department of Microbiology and Molecular Genetics, East Lansing, MI, United States

Gametocytes are the sexual stage of *Plasmodium* parasites responsible for malaria transmission from human to mosquito host. However, risk factors for gametocytemia such as age and ameliorating effects of interventions (antimalarial and ITN use) are poorly understood. We measured the prevalence and density of *P. falciparum* gametocytes by pfs25 gametocyte mature stage V marker using real-time quantitative nucleic acid sequence-based amplification assay (QT-NASBA) and by pfg377 female gametocyte marker using qRT-PCR on samples from a cross sectional survey conducted at peak transmission season in 2012 in Siaya County of western Kenya. We also measured parasitemia using 18S QT-NASBA. Total of 446 samples (225 malaria smear-positive and 221 randomly selected smear-negative) from 832 individuals were used to determine gametocyte carriage. Overall, 18S-NASBA detected 354 positives, of which 129 infections were from smear-negative individuals. Pfs25 was detected in 78.7% of smear positive and 10.9% of smear negative samples while pfg377 was found in 55.1% of smear positive and 2.7% of smear negative samples. In multivariable analysis, children (<5 and 5-15 years old) were more likely positive with pfs25 and pfg377 than adults >15 years old (pfs25: OR 3.4, CI 2.0-6.0 and OR 4.0, CI 2.1-7.7; pfg377: OR 15.3, CI 6.3-37.1 and OR 7.6, CI 3.0-19.3, respectively). Children <5 years were more likely pfg377 positive than children 5-15 years old (OR 2.0, CI 1.2-3.4); however, gametocyte density detected by pfg377 or pfs25 did not differ between these two age groups of children. Anemia (Hb < 11 g/dl) was associated with higher 18S density (1.45x per log10, CI 1.18-1.80, p=0.0005). Importantly, anemia was also associated with pfs25 and pfg377 positive status (OR 2.1, CI 1.4-3.4 and OR 2.4, CI 1.4-3.9). The odds of being pfs25 positive were lower in individuals using ITNs (OR 0.41, CI 0.23-0.71). No differences were seen for pfs25 density between individuals with and without ITN use. Antimalarial use (90% artemether-lumefantrine) during the two weeks prior to the survey was associated with fewer pfs25 carriers (OR 0.32, CI 0.17-0.62), but not with pfs25 density. These results show that children provided the highest gametocyte reservoir compared to adults and anemia was associated with an increased risk of gametocytemia. ITN and antimalarial use decreased the gametocyte prevalence, but not the gametocyte density in the study population.

1535

IDENTIFYING FACTORS ASSOCIATED WITH MALARIA PARASITEMIA IN MOZAMBIQUE USING A GEOSTATISTICAL MODEL

James Colborn[1], Kathryn Colborn[2], Edward Wenger[2], Guidion Mathe[3], Graca Matsinhe[3]

[1]Centers for Disease Control and Prevention, Maputo, Mozambique, [2]Intellectual Ventures, Seattle, WA, United States, [3]National Malaria Control Program, Maputo, Mozambique

Malaria is among the largest contributors to child mortality in Mozambique. Donors such as the Global Fund and the US President's Malaria Initiative have been successful in controlling malaria by supporting national scale-up of interventions, though transmission remains high in many parts of the country. Malaria Indicator Surveys (MIS) and Demographic Health Surveys (DHS) are intermittent household surveys

aimed at collecting nationally representative demographic and health related data, including socio-economic status (SES), insecticide treated net (ITN) ownership and use, coverage with indoor residual spraying (IRS), and malaria parasitemia prevalence by rapid diagnostic test (RDT) for children 6-59 months of age. When examined independently, these surveys may not capture annual variation in malaria transmission. To overcome this, we combined both the 2007 MIS and the 2011 DHS data to capture the inherent spatial and temporal variation in environmental factors and malaria transmission. Using these data, we estimated the associations between relevant factors and interventions and cluster-level parasitemia prevalence using a geostatistical model. This model included a fixed factor for survey year, IRS coverage, ITN use, age, SES (wealth quintiles), environmental factors, and a spatial random effect. Our results suggested that higher SES (OR=0.79; 95% CI 0.73, 0.85) and coverage of IRS up to 40% (OR=0.93; 95% CI 0.88, 1.00) were associated with decreased odds of parasitemia, and higher monthly average enhanced vegetation index (increased greenness; OR=5.10; 95% CI 1.83, 16.91) was associated with increased odds of parasitemia; ITN use was not associated with parasitemia, although this could be related to the low levels of ITN use in both survey years (cluster medians=0% and 33%, respectively). These findings suggest that socioeconomic status and vegetation are important factors to consider for understanding malaria transmission. Additionally, the lack of evidence using this analytic method for additional protection by IRS coverage above 40% bears further investigation.

1536

ASSESSING THE IMPACT OF CLINICAL MENTORSHIP ON MALARIA DIAGNOSIS AND TREATMENT PRACTICES IN UGANDA

Deepa K. Pindolia[1], Kathleen Maloney[1], Alex Ogwal[2], Chelsea Solmo[1], Vicky Abenakyo[2], Albert P. Okui[3], Anthony Mbonye[3], Bosco B. Agaba[3], Lindsay Bryson[1], Caitlin Dolkart[1], Justin M. Cohen[1], Arnaud Le Menach[1]

[1]Clinton Health Access Initiative, Boston, MA, United States, [2]Clinton Health Access Initiative, Kampala, Uganda, [3]Ministry of Health, Kampala, Uganda

To address problems associated with malaria misdiagnosis and inappropriate treatment, Uganda has implemented nationwide case management trainings, and procured Rapid Diagnostic Tests (RDTs) widely in the public sector since 2012. Despite these efforts, only 60% of suspected malaria cases were tested and 35% of negative tests were provided with anti-malarials in early 2014. In this study, a clinical mentorship for health workers, as a potential tool to overcome barriers of testing and adherence, and to improve overall fever case management is evaluated. This is a cluster randomized control trial, with one control and two intervention arms: one with district-selected peer mentors and one with facility in-charges as mentors. Mentorships occur on a monthly basis over 6 months, starting in April 2014 in 150 public health facilities distributed in 17 pilot medium endemicity districts. Diagnosis and treatment patient-level data are collected at monthly intervals in all study facilities. Multivariate logistic regressions will be performed to assess the impact of clinical mentorship on confirmatory diagnosis and on adherence to test results adjusted for covariates such as age, health facility level and district locations. At baseline, there were no significant differences in clinical diagnosis and adherence between any of study arms. Over 6 months, it is expected that clinical mentorship improves confirmatory diagnosis and adherence to test results in both intervention arms, and that differences of at least 10% are identified pre and post intervention and between each intervention arm and the control group. Clinical mentorship can work as an effective method to increase confirmatory diagnosis and adherence to test results, and improve overall fever case management, providing countries with a novel and effective tool to meet their national malaria case management targets.

1537

COLLABORATIVE DATA MANAGEMENT FRAMEWORK FOR BORDER MALARIA RESEARCH IN SOUTHEAST ASIA ICEMR CENTER

Ming-Chieh Lee[1], Ying Wang[2], Kirakorn Kiattibutr[3], Guofa Zhou[1], Jeeraphat Sirichaisinthop[4], Jetsumon Prachumsri[3], Liwang Cui[5], Guiyun Yan[1]

[1]University of California Irvine, Irvine, CA, United States, [2]Third Military Medical University, Chongqing, China, [3]Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, [4]Vector Borne Disease Training Center, Saraburi, Thailand, [5]Pennsylvania State University, University Park, PA, United States

Document and data archiving for the research activities are the tedious but important tasks through the whole scientific research project. It is a challenge for the scientist to maintain a solid, flawless and well organized data structure and data entry procedures especially for the project included international collaboration within multi-cultures and languages. Southeast Asia ICEMR center is focused on bolder malaria transmission between several Southeast Asia countries. We collaborated with several Universities, government agencies, local hospital and clinics, and field research labs in the remote border area of China, Myanmar, and Thailand. How to standardize the field survey and data entry procedures and semi-real-time to share research results is the goal for data manager and coordinators to conquer. Therefore, we design multi-language survey forms and data entry system as the tools to provide consistent interface and user experience for all research staffs. In order to expedite the data sharing and secured data services, we utilized several open source applications and cloud computing services to sustain our database system. To minimize the catastrophe of computer hardware/software collapse or network traffic congestion, the failover and load balancing features were setup in main server and several mirror servers and offsite remote backup scheme were implemented. To maintain the maximum data quality and structure, the standard operating procedures (SOPs) for field survey, data entry, data QA/QC were developed and documented. Currently the database system is online since July 2012.

1538

MALARIA PREVALENCE AND SPOROZOITIC INDEX IN SUBURBAN AREA IN KINSHASA BEFORE ITN MASS DISTRIBUTION, DR CONGO

Aimée Kahindo, Betricia Mavuzi, Freddy Kabasele, Serge M. Kayembe, Tommy M. Nseka, Solange E. Umesumbu, Thierry L. Bobanga

University of Kinshasa, Kinshasa, Democratic Republic of the Congo

Malaria is a parasitic disease due to *Plasmodium* transmitted by a female mosquito of *Anopheles* genus. It is a public health problem which causes a high mortality in less 5 years children. Estimated deaths by malaria word report are around 60000. This study interest is to update data on malaria prevalence in Kinshasa suburb area, in order to provide data for the stratification and control of the disease. The objectives of the study were to assess malaria prevalence into sub urban Kinshasa area population, to determine the most concerned population group, to identify plasmodial species and to determine sporozoitic index. An analytical cross-sectional survey was conducted in the village of LUZIZILA to 329 people whose age ranged between 6 months and 76 years for the period from August 10 to 25, 2013 Blood sample for a thick, a thin smear was conducted to determine the prevalence of malaria and determining plasmodial species. The sporozoitic index was determined by ELISA Results The overall prevalence of positive thick smears was 53.2%. In the age group under 5 years, prevalence was higher with 68.8%. Between 6 and 15 years it was 55% beyond 15 years the prevalence stood at 37.3%. *P. falciparum* was found in 97.7% of cases in thin smear slides The sporozoitic index was 11%. In conclusion, the prevalence found place Luzizila in an hyperendemic area. After the distribution we can expect a reduction of these indices

1539

MALARIA INFECTION IS ASSOCIATED WITH PREGNANCY LOSS IN OUELESSEBOUGOU, MALI

Almahamoudou Mahamar[1], Naissem Andemel[2], Youssoufa Sidibe[1], Adama Dembele[1], Moussa Traore[1], Oumar Attaher[1], Moctar Tounkara[1], Djibrilla Issiaka[1], Kadidia B. Cisse[1], Bakary S. Diarra[1], Moussa B. Kanoute[1], Amadou Barry[1], Patrick E. Duffy[2], Alassane Dicko[1], Michal Fried[2]

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

In malaria endemic areas, pregnant women are more susceptible to malaria infection compared to their non-pregnant counterparts. While the relationships between pregnancy malaria (PM) and outcomes such as severe maternal anemia and low birth weight are well established, there have been limited studies on the relationship between PM and pregnancy loss particularly in areas of high malaria transmission. We evaluated the relationship of fetal loss to malaria infection among pregnant women in Ouelessebougou Mali from November 2010 to January 2014. Pregnant women were enrolled during the antenatal consultation visits and followed up to delivery. Malaria infection in peripheral blood was detected by bloodsmear, and submicroscopic infection by PCR when the BS was negative. The proportion of women with submicroscopic malaria infection at delivery was 25.5% and pregnancy loss occurred in 5.8% of the cohort (80/ 1,377). Submicroscopic infection at delivery was associated with increased odds of fetal loss (unadjusted OR = 3.26, 95% confidence interval (CI) 1.35 - 7.89; and adjusted OR = 3.35, 1.37 - 8.16). A recent positive bloodsmear also increased the odds of fetal loss. In summary, preliminary analysis indicates that a submicroscopic malaria infection is associated with four times increase in odds of the pregnancy loss.

1540

ASYMPTOMATIC INFECTIONS AND MALARIA TRANSMITTED BY BLOOD TRANSFUSION: AN INVISIBLE RISK

Giselle F. Lima[1], José E. Levi[1], Maria E. Carvalho[2], Juliana Inoue[1], Angélica D. Hristov[1], Luis G. Nascimento[2], Arianni R. Sanchez[1], Eduardo M. Ramos-Sanchez[1], Maria J. Costa-Nascimento[2], Silvia M. Di Santi[1], Maria Carmen A. Sanchez[1]

[1]São Paulo University, São Paulo, Brazil, [2]SUCEN, São Paulo, Brazil

Transfusion transmitted malaria represents a major challenge, essentially due to the occurrence of asymptomatic infections. The vector transmission in Brazil mainly occurs in the Amazon Region, where 166,864 cases were notified in 2013. Outside the endemic region sporadic cases of autochthonous malaria are reported, including asymptomatic carriers of *Plasmodium*. In the state of São Paulo, transfusional cases were detected, due to asymptomatic donors harboring *P. malariae*, one of them leading to the death of the recipient. The occurrence of parasitemia without clinical symptoms in addition to the fact that *Plasmodium* can survive in stored red blood cells between 2 and 6º C for up to three weeks, increases the risk of transmission. In order to minimize the possibility of transfusional cases, the use of platforms including molecular and serological tests might point out donors suspected of harboring *Plasmodium*. We tested samples from 56 candidates for blood donation living in an area of São Paulo State where asymptomatic infections are reported. Thick blood smear, PCR, ELISA with recombinant *P. vivax* MSP119 antigen and SD Bioline Malaria Pf/Pv immunochromatographic test were used. Two samples (3.5%) (0.98 -12.1) were positive by thick blood film for *Plasmodium*, in a very low parasitemia. One of them was also positive by PCR, indicating the presence of *P. malariae*. ELISA detected 53.6% (40.7- 65.9) of samples reagent for *P. vivax*, with Reactivity Index ≥1.0. SD Bioline detected antibodies against *P. vivax* MSP and CSP recombinant antigens in 48.2% (35.6 - 60.9) of the samples. The frequency of positive samples in the serological tests pointed out to the risk of transfusional malaria, even in areas of low endemicity, since asymptomatic donors could be accepted based on clinical screening. Moreover, the lack of knowledge about this silent malaria outside the Amazon Region increases the risk of transmission. The use of platforms with different approaches could minimize this invisible risk.

1541

SUB PATENT INFECTION OF *PLASMODIUM FALCIPARUM* IN NORTHWESTERN PERU

Juan Jose Contreras-Mancilla[1], Dionicia Gamboa[2], Angel Rosas-Aguirre[1], Alejandro Llanos-Cuentas[1], Socrates Herrera[3]

[1]Instituto de Medicina Tropical Alexander von Humboldt, Lima, Peru, [2]Instituto de Medicina Tropical Alexander von Humboldt, Departamento de Ciencias Celulares y Moleculares, Facultad de Ciencias, Universidad Peruana Cayetano Heredia, Lima, Peru, [3]Latin American Center for Malaria Research, Cali, Colombia

The North-western region in Peru, is an area categorized as low endemicity for malaria after the El Niño Southern Oscillation (ENSO) phenomenon that increased the number of malaria cases to a peak with more than 200 000 cases in 1998 for both species, *Plasmodium vivax* and *P. falciparum*. The new treatment scheme implement in 2000 in this area decreased the number of *P. falciparum* cases in this area and since 2008, no cases have been reported by Ministry of Health. In Peru, microscopy is the diagnostic test used as routine by the Ministry of Health; but there are other technics as the Polymerase Chain Reaction (PCR), which is more sensitive to detect and identified correctly the species of *Plasmodium*, under the microscopy detection limit. In June of 2013, a total of 750 individuals from 3 urban areas were enrolled in a surveillance study in Piura, a malaria endemic region North-western of Peru: 350 from Bellavista, 329 from Obrero and 71 from Querecotillo. From each individual, a blood sample was taken to prepare 2 slides for microscopy and a filter paper for PCR diagnostic. Microscopy diagnosis was performed twice, one a local level and the second one by an expert microscopist as quality control. The DNA extraction from the filter paper was done by the Chelex-100 method and the PCR was based in a Real time protocol using specific probes to detect *P. falciparum* and/or *P. vivax*. No malaria cases was detected by microscopy; but PCR detected two positive cases for *P. falciparum* only, one case was located in Obrero and the other one in Bellavista. The parasitaemia level in both cases was lower than 450 parasites/µL and no symptom was present at moment when the sample was taken. These results showed the presences of *P. falciparum* in the North-western region of Peru and stress out the need to implement more sensitive tools for malaria diagnostic in areas of low endemicity where microscopy cannot detect if the country aims to improve control measures looking into malaria elimination.

1542

IMPLICATIONS AND EFFECTS OF DIVERSE *PLASMODIUM VIVAX* RELAPSE DISTRIBUTIONS IN SIMULATIONS OF VARYING TRANSMISSION SETTINGS

Philip A. Eckhoff, Caitlin A. Bever, Jaline Gerardin, Edward A. Wenger

Institute for Disease Modeling, Bellevue, WA, United States

Control and elimination of *Plasmodium vivax* is complicated by recurring relapses from hypnozoites in the liver of infected individuals. Different strains of vivax exhibit different patterns of relapse, ranging from the Chesson strain with an initial infection followed by early relapses, to strains with mixes of short and longer relapses, to North Korean strains that have infrequent early infections and longer latencies to relapse. Which strains predominate in a given geographic region depends on the local transmission setting, and earlier observational and modeling studies by various groups have allowed classification of different malaria zones. We present a new model for *P. vivax* transmission, host interactions, and relapse distributions and incorporate it into the EMOD model for malaria transmission. The broad diversity of relapse patterns is recreated with

a simple set of biological and immunological mechanisms, providing a mechanistic mathematical framework for comparing different strains. The fitness and population-level effects of different relapse patterns are then explored for a variety of transmission settings, with reference to earlier work by others on classification of malaria zones. Finally, implications of different relapse patterns for control and elimination efforts scaling up in the Solomon Islands and other settings are explored in simulation. Interventions simulated include primaquine and chloroquine combinations and vector control.

1543

NONINVASIVE SURVEILLANCE OF ZOONOTIC MALARIA

Josephine Siregar[1], Christina Faust[2], Lydia Murdiyarso[1], Andrew Dobson[2]

[1]Eijkman Institute of Molecular Biology, Jakarta, Indonesia, [2]Princeton University, Princeton, NJ, United States

The fifth human malaria parasite, *Plasmodium knowlesi*, is a novel public health threat in Southeast Asia. The parasite is primarily found in macaques, but within the last decade it has been increasingly recorded in humans, particularly on the island of Borneo. Human malaria treatment is effective for the parasite in humans, but to prevent transmission in the first place a better understanding of prevalence in its natural hosts is necessary. The objectives of this research are to develop and optimize noninvasive sampling methods for macaque malaria in wild populations. By using naturally infected macaques, we will compare blood and fecal samples to determine if noninvasive samples offer a logistical solution to widespread surveillance of macaque malarias. Results of this project will be applied to field collected specimens and inform experimental designs for surveillance of this pathogen in Borneo and elsewhere. Results from this work are essential for understanding malaria prevalence in macaque hosts and controlling emergence of the pathogen in new human populations.

1544

A COMPARATIVE CASE CONTROL STUDY OF THE DETERMINANTS OF CLINICAL MALARIA IN THE GAMBIA

Kalifa A. Bojang[1], Julia Mwesigwa[1], Eugene L. Kama Kama[2], Serign J. Ceesay[1], Serign J. Ceesay[1], Fanta Njie[1]

[1]MRC Unit, Banjul, Gambia, [2]National Institute of Public Health, Conakry, Guinea

The massive deployment of life saving malaria interventions has not only resulted in a decline in disease burden but a change in the risk of infection and disease. The study reassesses the importance of known risk factors and reviews socio-demographic determinants of malaria risk in the population. We conducted a case-control study involving 150 children aged 6 months to 12 years with slide-confirmed malaria recruited from the outpatient clinics of three health facilities (cases) in the Greater Banjul area, The Gambia. One hundred and fifty controls were matched on age, residence, and were negative for malaria. We collected information on the use of long lasting insecticidal nets, occupation of parents, housing structure, knowledge of malaria and socio-demographic factors. The mean age of study participants was 6.8 (SD 3.3) years with 147 (49%) being males. Significant determinants of malaria risk were parent's occupation: mother as trader (OR 0.18, 95% CI 0.04 - 0.73, p = 0.017), father as trader (OR 0.02, 95% CI 0.002- 0.193, p = 0.001), civil servants (OR 0.04, 95% CI 0.008- 0.257, p =0.001) or handyman (OR 0.03, 95% CI 0.005- 0.182, p < 0.001). Children sleeping in rooms with windowpanes had a 76% reduction in their odds of malaria (OR 0.24, 95%CI 0.07-0.82, p = <0.022. Household socio-economic status plays an important role in management of illnesses. The ability of mothers to engage in an occupation increases household resources to access healthcare and on time. The balance between the type of mother's occupation and her time available to supervise the child is an interesting emerging issue that needs further investigation.

1545

ANTIBODIES TO *PLASMODIUM VIVAX* MSP1-19 RECOMBINANT ANTIGEN IN BLOOD DONORS FROM BRAZILIAN LOW ENDEMIC AREAS

Arianni R. Sanchez[1], Eduardo M. Ramos-Sanchez[1], Silvano Wendel[2], Silvia M. Di Santi[1], Hiro Goto[1], Maria Carmen A. Sanchez[1]

[1]São Paulo University, São Paulo, Brazil, [2]Sírio Libanês Hospital, São Paulo, Brazil

In non-endemic and low endemic areas, transfusion-transmitted malaria (TTM) is a rarely reported event. However, four TTM were detected in São Paulo State, in Southeastern Brazil, including one death. Infected donors were identified as asymptomatic carriers with displacements to the Atlantic forest biome in São Paulo State. Due to the immune status of these donors, the parasite densities are low and undetectable in the thick blood smear or rapid diagnostic tests requiring the use of other methods for detection in blood banks outside the endemic areas. In this study, since *Plasmodim vivax* is the most prevalent species in Brazil, we assessed the prevalence of anti- *P. vivax* MSP1-19 IgG antibodies among blood donors from Southeastern Brazil. Initially, for validation, ELISA-PvMSP1-19 was assayed with 197 sera from patients with positive thick-blood smear for *P. vivax* yielding 96.95% sensitivity. A specificity of 100.0% was achieved in serum specimens from 101 normal individuals and 98.21% in 168 serum specimens from other diseases patients. After validation, 1,974 blood bank serum samples were tested: 1,309 from São Paulo and 665 from Rio de Janeiro. These samples were collected after the donors had been screened by clinical parameters, provided they were considered fit to donate and had signed the informed consent form. Regarding samples from São Paulo, 1.15% (N=15) positivity was achieved. In Rio de Janeiro samples, the positivity was 1.65% (N=11). The reactivity index (RI) of the positive samples ranged from 8.98 to 1.16 (Sao Paulo) and 13.03 to 1.08 (Rio de Janeiro). The detection of specific antibodies is not necessarily a marker of parasitemia or disease, but the detection of anti- *P. vivax* IgG antibodies in blood bank donors in non-endemic areas constitutes an alert that impel us to review the adopted criteria for screening of the donors aiming to reduce the risk of TTM in these areas without losing donations.

1546

HOUSEHOLD-LEVEL SOCIAL AND ENVIRONMENTAL FACTORS ASSOCIATED WITH BED NET OWNERSHIP AND DIFFERING MALARIA PREVALENCE IN SOUTHERN MALAWI

Lindsay R. Townes[1], Dyson Mwandama[2], Don P. Mathanga[2], Mark L. Wilson[1]

[1]University of Michigan, Ann Arbor, MI, United States, [2]Malaria Alert Centre, Blantyre, Malawi

Since 2002, Malawi's primary malaria prevention program has been a nationwide, health facility-based distribution of insecticide-treated nets (ITNs). Despite these efforts, ITN ownership in households with under-5 children remains sub-optimal. Knowing what characterizes such low-ownership households may improve targeting of ITN distribution. From June to August 2011, data on household ITN ownership, child malaria status, house location, building materials, and nearby environmental characteristics were collected by cross-sectional survey from 398 households in two rural Traditional Authorities (TAs) of southern Malawi, Sitola and Nsamala, which were in the catchment area of Machinga District Hospital (MDH). The proportion of households in Sitola reporting bed net ownership was significantly lower (OR 0.59, 95% CI 0.39-0.89), and the prevalence of malaria among under-5s significantly higher (OR 4.20, 95% CI 2.74-6.46) than in Nsamala, this despite higher bed net use among owners in Sitola (OR 2.56, 95% CI 1.00-6.57). Households in Sitola were also much more likely to be located within 50 m of active agriculture (OR 9.60, 95% CI 4.93-18.68), of brick-making sites (OR 4.59, 95% CI 2.34-9.01), and of water sources (OR 11.87, 95% CI 3.49-40.40). Households in the two TAs did not differ with respect to housing materials,

recent or current maternal pregnancy, number of children, or number of household residents. Among those with a bed net, there was no difference in whether they had received their bed net at MDH or whether the net was insecticide-treated. Curiously, within the higher malaria prevalence context of Sitola, ITN ownership was not significantly associated with child malaria status, land use/land cover, quality of housing materials, nor recent maternal pregnancy; however, current maternal pregnancy was inversely associated with net ownership (OR 0.35, 95% CI 0.13-0.95). In contrast, current maternal pregnancy had no association with net ownership in Nsamala (OR 0.69, 95% CI 0.31-1.50), while houses built with higher quality materials were more likely to own at least one bed net (OR 3.31, 95% CI 1.20-9.12). These results suggest a geographical disparity in ITN distribution between Nsamala and Sitola, which may be reduced through improved targeting of pregnant women in Sitola.

1547

MALARIA TRANSMISSION IN BOLENGE HEALTH ZONE, EQUATORIAL SETTING, DR CONGO

Thierry L. Bobanga[1], Billy S. Kunyu[2], Solange E. Umesumbu[3], Olivier A. Fataki[1], Prosper Abanda[4], Dieudonné N. Mumba[1]

[1]University of Kinshasa, Kinshasa, Democratic Republic of the Congo, [2]National Institute of Biomedical Research, Kinshasa, Democratic Republic of the Congo, [3]National Malaria Control Program, Kinshasa, Democratic Republic of the Congo, [4]Bolenge Health Zone, Mbandaka, Democratic Republic of the Congo

Malaria constitutes a public health major problem in Democratic Republic of Congo (DRC). For malaria control , one of adopted approach is Insecticide treated Net(ITN). In order to evaluate the malaria transmission, level in a area , different parameters are therefore used. One of them is the parasitic parameter which includes plasmodic index (PI), gametocytic index (GI), parasitemia density (PD) and the plasmodial species. Another parameter is the sporozoitic index (SI). This study aimed to assess the level of transmission in a stable Heath Zone where ITN have been partially distributed. A transversal study has been conducted from 11th of October to 17th of November 2011 in Bolenge Heath Zone where in, there are 3 Health Areas(Bolenge, Wendji Secli and Bongonde) separated between themselves by the distance of at least 10 kilometers, have been selected. Thick blood smear and thin blood smear have been done in all members of the households which remount to 185 in total, which include by the way 1066 subjects. *Anopheles* were captured in household for determining SI Results The global PI in Bolenge Health zone was of 41.8 %. The rate of mosquisto bednet utilization was 95 %, 13 % and 23 %, respectively in Bolenge health area, Bongonde and wendji-Secli and in the same way, the PI was of 32.7 %, 50.4 % and 42.2 %; p<0.01. The global average parasitemia of 3 Heath areas was of 2213±354 trophozoites/ µl (2326.±54; 3182±603 and 965.±194 respectively in Bolenge, Bongonde and Wendji-Secli health areas and in the same way, GI was of 3.7 %, 10.4 % and 4.4 % SI was respectively 5, 7 et 10 in Bolenge , Wendji secli and Bongonde and..*Plasmodium falciparum* was found at 99.9 %. All anopheles were An.gambiae s.s M molecular form. In conclusion, transmission was high in Bolenge Health Zone, it was very raised in Bongonde Health Area, where the rate of the ITN use was low.

1548

DETERMINANTS OF RISK OF MALARIA PARASITEMIA IN BUNKPURUGU-YUNYOO DISTRICT, NORTHERN GHANA, INCORPORATING REMOTE SENSING AND SURVEY DATA

Paul J. Psychas[1], Brooke Eckman[1], Benjamin Abuaku[2], Collins Ahorlu[2], Kwadwo Koram[2]

[1]University of Florida Emerging Pathogens Institute, Gainesville, FL, United States, [2]Noguchi Memorial Institute of Medical Research, University of Ghana, Legon, Ghana

Ghana's malaria control strategy prioritizes the northern savannah regions due to persistent hyperendemicity. In Bunkpurugu-Yunyoo district, previously reported anemia and parasitemia surveys were conducted serially in 3 rainy seasons (RS) and 3 dry seasons (DS) in 2010-13, covering 11,945 children under five from 179 communities. In spite of high coverage for insecticide-treated bed nets (>75% use in each RS) and indoor residual spraying (IRS) with pyrethroid pesticides in years 2 and 3 (>98% households sprayed), investigators found unexpectedly high and geographically heterogeneous malaria prevalence. To better define and explain this heterogeneity, this study enhanced the survey dataset with remotely sensed data, then analyzed by ecologic zones, delineated as urban (Zone 1, n=1131); rocky uplands (Zone 2, n=5234, >750 ft altitude); transition (Zone 3, n=3456), and riverine plains (Zone 4, n=2124, <550 ft). The RS odds ratios for microscopic malaria parasitemia in children living in Zones 2, 3, and 4, as compared with Zone 1, were respectively 3.9 (95% CI: 2.8-5.4), 7.6 (95% CI:5.7-10.3), and 11.1 (95% CI: 8.0-15.6; all p values here and in the following <0.0001). Zone 4 parasitemia prevalence across the 3 years was 65.6-72.1% in the RS and 39.8-54.9% in DS. Among 17 variables with statistically significant odds ratios (OR) for malaria risk, 12 exhibited a zonal gradient favoring reduced risk in Zone 1 vs. Zone 4, with Zone 2 intermediate. In the RS these included lower wealth quintile (OR=3.6; 95% CI:2.7-4.7), caregiver's lack of education (OR = 2.7; CI: 2.1-3.3), ethnicity (OR = 3.9; CI: 3.2-4.8), lack of health insurance coverage (OR 3.0; CI=2.4-3.6), higher vegetation index (OR=1.6; CI: 1.1-2.3), lower human influence index (OR 5.2; CI:3.8-7.1); and >3 km distance to nearest health facility (OR=2.4; CI:1.9-3.1), among others. DS findings were similar. No consistent zonal gradient was found for the malaria control measures (ITN use, ACT use, IRS). Findings suggest that, in spite of high coverage with ITNs and pyrethroid-based IRS, high malaria prevalence in northern Ghana may be found in locations where reduced socioeconomic status and isolation coincide with low-lying terrain. Such areas may require additional and/or modified methods for vector and parasite control.

1549

DEVELOPING A PASSIVE MALARIA CASE DETECTION STRATEGY IN TANZANIAN MILITARY HEALTH FACILITIES AND MALARIA EPIDEMIOLOGY DATA COLLECTED TO DATE

Akili Kalinga[1], Christopher Mswanya[2], George Amoo[3], Charles Mwanziva[4], Sarah Chiduo[5], Lucky Temu[5], Lalaine Anova[6], Colin Ohrt[6]

[1]National Institute of Medical Research, Dar es Salaam, United Republic of Tanzania, [2]Tanzania Peoples Defense Forces, Dar es Salaam, United Republic of Tanzania, [3]Amethyst Technologies LLC, Baltimore, MD, United States, [4]Tanzania Peoples Defense Force, Dar es Salaam, United Republic of Tanzania, [5]Walter Reed Malaria Programme-Tanzania, Dar es Salaam, United Republic of Tanzania, [6]Walter Reed Army Institute of Research, Silver Spring, MD, United States

The capability to accurately track malaria incidence is essential to measuring the true impact and efficacy of malaria control interventions or field evaluations of malaria therapeutics, vaccines, etc. However, gathering reliable malaria epidemiology data in rural and resource-challenged settings is often a daunting and difficult endeavor. In Tanzania, malaria persists as a major cause of morbidity and mortality. The US Army has partnered with the Tanzania People's Defence Forces (TPDF) and the Tanzania National Service Program (JKT) to support the TPDF's efforts to improve malaria management in a number of TPDF and JKT camps. The TPDF is an important provider of health services for both military and civilian populations, especially in remote areas where TPDF camps are based. Our initial efforts to perform passive malaria case detection relied on collecting microscopy slides from sites for cross-checking. These efforts were resource intensive and resulted in dubious success and questionable data. This led us to transition our focus to the use of malaria rapid diagnostic tests (RDTs) with RDT readers. In our approach, we deployed the Deki Reader, a rugged, mobile *in vitro* diagnostic device which interprets commercially available RDTs. Several advantages provided by the Deki system include real-time quality control measures, the ability for remote quality assurance (QA), and the automatic organization of

the data in a centralized web-based portal. After deploying devices to each site, training is provided to site staff regarding use of RDTs and Deki Readers and sites are allowed a period of practice. After the sites become active, QA monitors review the mRDT database and conduct QA quarterly visits for trouble-shooting, to cross-check mRDT results against laboratory, physician, and pharmacy records, and technician compliance and accuracy for data transmission. In our first year of implementation, our approach has undergone several adjustments to adapt to a number of challenges, however we have made great strides improving the reliability of the data. We will present the challenges and successes experienced with implementation of our approach and the data collected to date.

1550

PRECISE GENOTYPING TOOLS FOR INVESTIGATING TRANSMISSION DYNAMICS OF *PLASMODIUM FALCIPARUM* GAMETOCYTES

Rahel Wampfler[1], Lincoln Timinao[2], Hans-Peter Beck[1], Issiaka Soulama[3], Alfred B. Tiono[3], Peter Siba[2], Ivo Mueller[4], Ingrid Felger[1]

[1]Swiss Tropical and Public Health Institute, Basel, Switzerland, [2]Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea, [3]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso, [4]Walter and Eliza Hall Institute, Parkville, Australia

Differentiation between gametocyte-producing *Plasmodium falciparum* clones depends on high stage-specific expression and high genetic diversity of a genotyping marker in the study area. High-resolution typing methods are crucial for longitudinal tracking of gametocyte producing clones in multiple infections. Pfs230 and pfg377 are classical length-polymorphic markers for differentiation of gametocytes. We have evaluated capillary electrophoresis-based differentiation of 6 length-polymorphic gametocyte genes. These assays were applied to asexual parasites by targeting genomic DNA from field samples and in parallel to gametocytes from the same blood samples by targeting gametocyte-specific RNA. Highest diversity was found for pfs230 with 18 alleles and for pfg377 with 15 alleles in 111 samples from PNG. When assays were performed in parallel on RNA and DNA from 46 samples from Burkina Faso, 85.7% of all pfs230 samples and 59.5% of all pfg377 samples contained at least one matching genotype in DNA and RNA. Out of the 93 PCR fragments amplified from DNA of all samples by pfs230, 41 (44.1%) were not observed in the corresponding RNA sample. Vice versa we found that 42.9% (39/91) of pfs230 fragments detected in RNA failed to be amplified from the corresponding DNA samples. The imperfect detection in both, DNA and RNA, was identified as major limitation for investigating transmission dynamics, owing primarily to the volume of blood processed and the incomplete representation of all clones in the sample tested. This finding emphasises the importance of expression levels of gametocyte-specific markers as well as optimal sampling and preservation of DNA and RNA. Larger volumes may improve clone detectability of abundant low-density gametocyte carriers and of initially sequestered gametocyte clones in follow-up samples. Application of these methods to samples from cohort studies will help to explain additional factors influencing detectability of gametocyte clones, e.g. the dynamics of gametocytogenesis of a specific parasite clone over the duration of its infection.

1551

CAN HEALTH MANAGEMENT INFORMATION SYSTEM (HMIS) DATA BE USED TO MAKE INDOOR RESIDUAL SPRAY POLICY DECISIONS IN BENIN?

Evelyne S. Alyko Chaffa[1], Elana Fiekowsky[2], Joel Sodjinou[1], Benjamin Johns[2], Allison Belemvire[3], Kristen George[3], Christen Fornadel[3]

[1]Abt Associates, Cotonou, Benin, [2]Abt Associates, Bethesda, MD, United States, [3]United States Agency for International Development, Washington, DC, United States

The President's Malaria Initiative (PMI) has supported Indoor Residual Spraying (IRS) in Benin as part of its malaria control strategy. The PMI/USAID-funded Africa Indoor Residual Spraying (AIRS) Project assessed the feasibility of using health facility data to inform targeted spray decision-making, and assess IRS's effect on malaria caseloads. AIRS accessed routinely HMIS data from nine IRS intervention districts and five neighboring comparison districts, covering the period 2006 - 2013, including the period of IRS implementation from 2011-2013. We then assessed the association between IRS and the number of malaria cases reported by health facilities for both the total population and children under five years of age. Two models were developed for this assessment. In the first model, we first assessed, for a subset of areas with available data, the association of IRS on the Entomological Inoculation Rate (EIR), we then analyzed the association between EIR and the number of malaria cases as reported in the HMIS data. Finally, we combined these two steps to evaluate the association of IRS on reported malaria cases through the EIR. The second model measured the association of IRS directly on reported malaria cases in the HMIS data. The sample size for the first model was small, yet it serves to assess the validity of the second model. For the analyses, we used a difference-in-difference approach that controls for rainfall, other malaria and health interventions, and time trends. We assessed the internal validity of the data assessing the association of other malaria interventions on health facility utilization and putting the treatment on a year before IRS took place to measure the association of a "false" treatment in the second model. We assessed the association separately for two classes of insecticides, carbamates and organophosphates. As estimated against the comparison group, initial results show a 20 to 30 percent decrease in the number of confirmed cases per person per month associated with carbamates and a 5 to 20 percent decrease per person per month associated with organophosphates. The sensitivity tests and the falsification tests make us question the internal validity of the second model; the falsification test showed a statistically significant effect in a year that IRS did not take place. Based on this research, we cannot recommend using HMIS data to make IRS targeting policy decisions in Benin without further data validation.

1552

INVESTIGATING THE SPECIFICITY AND KINETICS OF *PLASMODIUM FALCIPARUM*-SPECIFIC IGG RESPONSES THAT ASSOCIATE WITH PROTECTION FROM MALARIA: A LONGITUDINAL STUDY IN MALI

Eugene Liu[1], Jeff Skinner[1], Tuan M. Tran[1], Aissata Ongoiba[2], Shanping Li[1], Safiatou Doumbo[2], Didier Doumtabe[2], Younoussou Kone[2], Aboudramane Bathily[2], Seydou Dia[2], Moussa Niangaly[2], Jules Sangala[2], Chiung-Yu Huang[3], Huw Davies[4], Aarti Jain[4], Arlo Randall[5], Ogobara K. Doumbo[2], Kassoum Kayentao[2], Boubacar Traore[2], Philip L. Felgner[4], Peter D. Crompton[1]

[1]National Institutes of Health, Rockville, MD, United States, [2]Mali International Center of Excellence in Research, University of Sciences, Technique and Technology of Bamako, Bamako, Mali, [3]Research Program in Quantitative Sciences, Division of Oncology Biostatistics/Bioinformatics, The Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD, United States, [4]University of California Irvine, Irvine, CA, United States, [5]Antigen Discovery Inc., Irvine, CA, United States

Antibodies play a key role in malaria immunity, but antibody-mediated protection is only acquired after years of *Plasmodium falciparum* exposure, leaving children vulnerable to repeated bouts of symptomatic malaria. Our previous work in Mali suggests *P. falciparum* protein microarrays can be used in population-based studies to investigate the antigen specificity of protective antibodies and the kinetics of their acquisition; however, our prior work was limited by small samples size, a lack of active surveillance for clinical malaria and *P. falciparum* infection, and few time points. To address these limitations we conducted an independent two year cohort study in the rural village of Kalifabougou, Mali where intense malaria transmission occurs from June to December. Active surveillance for clinical malaria and *P. falciparum* infection was done weekly and bi-weekly, respectively. Of the 695 enrollees in the cohort study (aged 2 months to 25 years), we focused the present analysis on the 268 subjects who were *P. falciparum* PCR negative at enrollment before the malaria season. A microarray with 1024 *P. falciparum* proteins was probed with plasma collected from these 268 subjects at four time points: before the six-month malaria season, during the first episode of febrile malaria of the ensuing malaria season (if it did occur), after the malaria season, and after the subsequent 6-month dry season. In ongoing analyses that we expect to complete by mid 2014, we are comparing antibody profiles of children who were prospectively classified as clinically immune (documented infection not followed by fever) or susceptible to malaria, as well as individuals who showed evidence of sterile protection. We are also modeling the breadth, magnitude and kinetics of *P. falciparum*-specific antibody responses from 2 months to 25 years--the age range over which clinical immunity to malaria is acquired in this population. This rich dataset is shedding light on fundamental properties of the human antibody response to malaria and may help identify novel malaria vaccine targets.

1553

CIRCULATING IMMUNOGLOBULIN (IGG) AGAINST MSP1-$_{42}$ AND *PF*EMP-1 ARE NOT ASSOCIATED WITH PEDIATRIC MALARIA SEVERITY IN WESTERN KENYA

Evans Raballah[1], Prakasha Kempaiah[2], Samuel B. Anyona[1], Zachary Karim[2], Mark Webale[1], George Orinda[3], Michael F. Otieno[4], James B. Hittner[5], Douglas J. Perkins[2], John M. Ong'echa[1]

[1]University of New Mexico Laboratories of Parasitic and Viral Diseases, Kisumu, Kenya, [2]Center for Global Health, Albuquerque, NM, United States, [3]Department of Biochemistry and Biotechnology, Nairobi, Kenya, [4]Department of Medical Laboratory Sciences, Nairobi, Kenya, [5]Department of Psychology, Charleston, SC, United States

Malaria remains a major cause of morbidity and mortality among naïve immune children and pregnant women of Africa. The majority of cases are caused by *Plasmodium falciparum*. In holoendemic regions such as western Kenya, severe malaria cases in children under the age of five years manifests as severe malarial anemia [Hemoglobin (Hb) <6.0g/dL; any density parasitemia]. High levels of Immunoglobulin (Ig)-G to a number of surface proteins and invasion ligands have been associated with protection from malaria. Morever, recent studies suggest that MSP1-$_{42}$ interacts with heparin-like molecules on the RBC. Adhesion is mediated by the *P. falciparum* erythrocyte membrane protein 1 (*Pf*EMP-1), expressed at the surface of infected erythrocytes and is linked to both antigenic variation and cytoadherence. The role of antibodies against these antigens in the pathogenesis of SMA remains largely unknown. We therefore sought to elucidate the role of these antibodies by measuring circulating IgG levels against these antigens in children (n=117) presenting with acute malaria at Siaya County Hospital, western Kenya. Complete hematological measures were obtained with a Beckman Coulter Counter®, and Giemsa-stained slides were used to determine parasitemia. Participants were stratified based on Hb status as non-SMA (n=91), Hb≥6.0 g/dL and SMA (n=26), Hb<6.0 g/dL. Results presented here show that circulating IgG against MSP1-$_{42}$ were comparable between non-SMA and SMA groups [Median (Interquartile range) non-SMA 128.83 (295) and SMA 151.19 (321); *P*=0.963]. In addition, the IgG levels against MSP1-$_{42}$ did not correlate with parasite density (ρ=0.065; *P*=0.487). Similarly, circulating IgG against *Pf*EMP-1 was comparable between the groups [Median (Interquartile range) non-SMA 420.0 (590) and SMA 377.97 (710); *P*=0.632] and was not associated with parasite density (ρ=-0.006; *P*=0.952). These results suggest that the levels of circulating IgG against MSP1-$_{42}$ and *Pf*EMP-1 are not correlated with malaria disease severity in acutely infected children from this holoendemic region.

1554

THE ROLE OF THE INHIBITORY FC RECEPTOR, FCΓRIIB IN HOST IMMUNE RESPONSE AND SUSCEPTIBILITY TO MALARIA IN PAPUA NEW GUINEA

Alexander M. Barron[1], **Gloria Tavera**[1], Arlene E. Dent[1], Peter M. Siba[2], James W. Kazura[1], Christopher L. King[1]

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

Binding of human Fragment crystalizable gamma receptor 2b (FcγRIIb) on B cells to antigen (Ag)-containing immune complexes (ICs) mediates a critical inhibition of host immune responses. Homozygosity for a FcγRIIb missense mutation (p.Ile232Thr) that reduces inhibition is associated with protection against severe malaria in Kenyan children. Protection against severe malaria in p.232Thr/Thr (TT) Kenyan children may result from a more robust humoral immune response and/or enhanced phagocytosis of malaria-infected erythrocytes (iRBC), which may select for this mutation. This hypothesis is supported by observations that TT frequency is high in malaria endemic areas (5-11%) and low elsewhere (~1-3%). In Papua New Guinea, the frequency of I232T phenotypes did not significantly differ between groups living in different malaria endemic versus non-endemic areas (p = 0.3794), however preliminary analysis suggests that individuals with TT phenotype were less likely to be infected with *P. vivax* malaria (p = 0.0319, N = 544). Studies are underway to correlate TT phenotype with risk of malaria disease. To understand how the TT phenotype may mediate protection from malaria infection and disease, we examined malaria-specific antibody (Ab) levels among different FCGR2B genotypes since TT polymorphism reduces repression of B cell activating pathways. Unexpectedly, TT Papuans had smaller repertoires and lower levels of *P. falciparum*- and *P. vivax*-specific Ab than II Papuans (i.e. p = 0.0045 for Duffy Binding Protein). Studies are underway to determine the role of FcγRIIb in modulating phagocytosis, further elucidating its role in regulating host immune responses and susceptibility to malaria.

1555

UNRAVELING THE HUMAN IMMUNE RESPONSE TO SYMPTOMATIC AND ASYMPTOMATIC *PLASMODIUM VIVAX* INFECTIONS THROUGH SYSTEMS IMMUNOLOGY

Vitor R. Mendonca[1], Andrea Kuhn[2], Marcus V. Lacerda[2], Steve Porcella[3], Manoel Barral-Netto[4], Peter D. Crompton[1]

[1]National Institute of Allergy and Infectious Diseases, Rockville, MD, United States, [2]FMT-HVD, Manaus, Brazil, [3]National Institutes of Health, Hamilton, MT, United States, [4]FIOCRUZ-BA, Salvador, Brazil

Approximately 2-3 billion people are at risk of *Plasmodium vivax* infection worldwide. The nature of the immune response during symptomatic and asymptomatic *P. vivax* infection remains elusive. We are applying systems biology tools such as genome-wide expression profiling by RNA-seq and multi-parameter flow cytometry to gain insight into host immune responses that associate with protection from disease during *P. vivax* infection. In the Brazilian Amazon we enrolled three groups: 1) *P. vivax*-infected adults with fever (n=19), 2) *P. vivax*-infected adults with no fever or symptoms for 30 days despite persistent parasitemia (n=17), and 3) age-matched uninfected controls (n=17). Blood samples were collected at enrollment from the first and third groups, whereas blood was collected from the second group after the 30-day period without fever or symptoms. Standard hematology and chemistry labs were done; and plasma, peripheral blood mononuclear cells (PBMCs) and RNA were isolated from whole blood. Analysis of the hematologic data showed that symptomatic subjects had a higher percentage of neutrophils (median 74.1%, p=0.0002) and a lower percentage of lymphocytes (median 21.0%, p=0.0007) compared to asymptomatic subjects. Symptomatic subjects also had higher levels of total bilirubin, creatinine and glucose (p<0.0001 for each comparison) versus asymptomatic subjects. Purified RNA was converted to cDNA and sequenced by next generation sequencing. In ongoing analysis of the RNA-seq data, differentially expressed pathways and gene sets in immune and susceptible individuals will be confirmed at the protein level and functionally using contemporaneous PBMCs and plasma samples. Molecular and cellular signatures that correlate with protection from malaria fever may yield new hypotheses regarding the biological mechanisms by which malaria immunity is induced by *P. vivax* infection. The resulting datasets may be of considerable value in the urgent effort to develop a malaria vaccine.

1556

EVALUATING CONTROLLED HUMAN MALARIA INFECTION IN KENYAN ADULTS WITH VARYING DEGREES OF PRIOR EXPOSURE TO *PLASMODIUM FALCIPARUM* USING SPOROZOITES ADMINISTERED BY INTRAMUSCULAR INJECTION

Susanne H. Hodgson[1], Elizabeth A. Juma[2], Amina Salim[3], Charles Magiri[2], Domtila Kimani[3], Daniel Njenga[2], Alfred Muia[2], Andrew O. Cole[2], Caroline Ogwang[3], Ken Awuondo[3], Brett Lowe[3], Marianne Munene[3], Peter F. Billingsley[4], Eric R. James[4], Anusha Gunasekera[4], B. K. Sim[4], Patricia Njuguna[3], Thomas Rampling[1], Adam Richman[4], Yonas Abebe[4], Gathoni Kamuyu[3], Michelle Muthui[3], Sean C. Elias[1], Sassy Molyneux[3], Stephen Gerry[5], Alex Macharia[3], Thomas N. Williams[3], Peter C. Bull[3], Adrian V. Hill[1], Faith H. Osier[3], Simon J. Draper[1], Philip Bejon[3], Stephen L. Hoffman[4], Bernhards Ogutu[3], Kevin Marsh[3]

[1]University of Oxford, The Jenner Institute, Oxford, United Kingdom, [2]Centre for Clinical Research, Kenya Medical Research Institute, Nairobi, Kenya, [3]Kenya Medical Research Institute - Wellcome Trust, Centre for Geographical Medical Research (Coast), Kilifi, Kenya, [4]Sanaria Inc., Rockville, MD, United States, [5]University of Oxford, Centre for Statistics in Medicine, Oxford, United Kingdom

Controlled human malaria infection (CHMI) studies, where healthy volunteers are infected with *Plasmodium falciparum* have become a vital tool to accelerate vaccine and drug development. As CHMI trials are carried out in a controlled environment, they allow unprecedented, detailed evaluation of parasite growth dynamics and immunological responses to infection. However, to date CHMI studies have not been used to investigate mechanisms of naturally-acquired immunity (NAI) to *P. falciparum* infection. We conducted an open label, randomized CHMI study using aseptic, cryopreserved *P. falciparum* sporozoites (PfSPZ Challenge) administered intramuscularly to evaluate infectivity and parasite growth dynamics in healthy Kenyan adults (n=28) with varying degrees of prior exposure to *P. falciparum*. All participants developed blood-stage infection, however one volunteer remained asymptomatic and blood film negative until day 21 post injection of PfSPZ Challenge, despite developing confirmed blood-stage infection by quantitative polymerase chain reaction (qPCR). A significant correlation was seen between parasite multiplication rate (PMR) and anti-schizont ELISA OD at screening (p=0.044; r=-0.384). Our study has shown that CHMI using PfSPZ Challenge is safe in African adults who have varying degrees of prior exposure to malaria and that NAI can impact on PMR post-CHMI, providing a novel method to investigate the dynamics and mechanisms of blood-stage immunity.

1557

CYTOKINE RESPONSES TO THE VAR2CSA VACCINE CANDIDATE IN PREGNANT BENINESE

Komi Gbedande[1], Gilles Cottrell[2], Samad Ibitokou[1], Bertin Vianou[1], Carine Agbowaï[1], Marita Troye-Blomberg[3], Thor Theander[4], Ali Salanti[4], Achille Massougbodji[1], Kabirou Moutairou[5], Nicaise Tuikue Ndam[2], Philippe Deloron[2], Adrian Luty[2], Nadine Fievet[2]

[1]Centre d'Etude et de Recherche sur le Paludisme Associé à la Grossesse et à l'Enfance, Cotonou, Benin, [2]Institut de Recherche pour le Développement, UMR D216 Mère et enfant face aux infections tropicales, Paris, France, [3]Department of Immunology, Wenner-Gren Institute, Stockholm University, Stockholm, Sweden, [4]Department of International Health, Institute of International Health, Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark, [5]Faculté des sciences et techniques, Université d'Abomey-Calavi, Cotonou, Benin

The STOPPAM consortium conducted 2 longitudinal cohort studies in pregnant women in Benin and Tanzania in order to evaluate the immunopathological consequences of infections with *Plasmodium falciparum* during pregnancy. In this context, parasite antigen-specific cellular responses, in particular to the vaccine candidate antigen VAR2CSA, have received little attention. Here we evaluated both, cytokine (IL10, IL13, IL17, IFN- γ, TNF-α) responses and the T cells IFN-γ specific responses to the DBL5 domain of VAR2CSA. In Come, southwestern Benin, we conducted a longitudinal prospective study of ~1000 pregnant women. Women at ≤24 weeks of pregnancy were enrolled and followed at each antenatal visit until delivery. Peripheral blood mononuclear cellular (PBMC) responses to VAR2CSA-DBL5 *in vitro* were determined in subgroups of 150 women at inclusion and 100 at delivery. In each subgroup those harbouring *P. falciparum* infections were matched by gravidity and gestational age with mothers with no infection at inclusion and those with no history of infection earlier in the pregnancy. The amounts of IL10, IL13, IL17, IFN-γ and TNF-α produced in response to mitogen (PHA) and to VAR2CSA-DBL5 were quantified in supernatants of stimulated PBMC. The *ex vivo* frequencies of IFN-γ secreting CD4 and CD8 T cells in response to PHA and VAR2CSA domains were evaluated in the same maternal PBMC groups. At the time of writing, all data have been collected, cytokine concentrations have been evaluated and multivariate analyses are under way. Results will be discussed firstly in the context of cytokine profiles that reflect the acquisition of a specific cellular memory response to the vaccine candidate according to gravidity or to previous *P. falciparum* infection. Secondly, we will discuss cytokine profiles as potential markers of protection in the context of infection, anemia and birth weight.

1558

MFERA (MALAWI) COHORT STUDY: COMMUNITY-BASED LONGITUDINAL STUDY OF MALARIA IMMUNOLOGY, EPIDEMIOLOGY AND GENOMICS

Esther N. Gondwe

College of Medicine, Malawi, Blantyre, Malawi

Malaria is endemic in Malawi, with climatic factors supporting transmission in most of the country except at high elevation. Individuals at risk of malaria infection are mostly young children and pregnant women. The risk of severe malarial disease and clinical symptoms declines in children residing in endemic regions when they experience continuing exposure to infectious mosquito bites. Thus older children sustain only mild symptoms or are asymptomatic. To understand host responses that develop with repeated exposures and are associated with clinical disease immunity, we undertook a longitudinal cohort study at Mfera Health Centre, Chikhwawa district in Malawi. 120 subjects with uncomplicated malaria are being followed for two years to capture host responses during repeated infections. We will also examine host responses in three age groups (1-5 years, 6-12 years and 13-50 years), as age serves as a proxy for clinical immunity. We will present data by age group to include clinical symptoms, rate of recurrence, host response profiling and parasite genotypes. We will also analyse these features in individuals who have repeated infections to identify changes over time using repeated measures design. A primary focus of the host response studies is the role of type I IFN in the development of clinical immunity. The role of type I IFN to confer protection or susceptibility to clinical malaria remains controversial and thus we will focus on type I IFN responses (cytokine, interferon responsive genes, type I IFN receptor genetic variants, immunophenotyping of relevant cells) in association with disease markers and age. The data generated will provide an unprecedented opportunity to understand how residents of malaria endemic areas develop less severe clinical disease with repeated parasite exposures and these data may inform vaccine strategies.

1559

PD1 EXPRESSION ON NEONATAL VΔ2 T CELLS MODULATES FUNCTIONAL RESPONSES TO MICROBIAL ANTIGENS

Sarah E. Boudova[1], Suchita Chaudhry[2], Christopher Harman[1], Miriam Laufer[1], David Pauza[2], **Cristiana Cairo**[2]

[1]University of Maryland, School of Medicine, Baltimore, MD, United States, [2]University of Maryland, Institute of Human Virology, Baltimore, MD, United States

In utero exposure to microbial antigens primes the fetal immune system, often with negative consequences for infant immunity and responses to pediatric vaccines. VΔ2 T cells, a subset of γΔ T cells, play important roles in antimicrobial immunity and participate in responses to Bacille Calmette-Guérin (BCG) vaccination. We observed that prenatal exposure to plasmodium antigens primes fetal VΔ2 cells, potentially causing dysregulation of infant VΔ2 cells. We are defining the impact of prenatal *Plasmodium falciparum* exposure on neonatal and infant immunity by measuring changes in VΔ2 cells. In this context, we want to identify inhibitory and activating receptors that modulate VΔ2 responses and differ in expression on fetal versus adult cells. These differences in regulation are important for normal immune function and may be altered by maternal infections. PD1 is a key negative regulator of immune responses and a marker of T cell functional exhaustion during chronic viral infections and malaria. We compared PD1 expression on healthy North American adult and neonatal VΔ2 cells after stimulation. For adult VΔ2 cells, PD1 expression peaked by day 4 and in most individuals returned to baseline by day 14. For neonatal (cord blood) VΔ2 cells, PD1 expression peaked between days 4 and 7, and in most subjects was still elevated at day 14, yielding a PD1+ fraction significantly larger than in adults (43.6% versus 8.5%, $p<0.0001$). PD1 expression on neonatal VΔ2 cells remained stable up to day 35. The ability of neonatal VΔ2 cells to produce the pro-inflammatory cytokine TNFα and mobilize cytotoxic granules in response to immobilized anti-T Cell Receptor antibody was inhibited by immobilized PD1-ligand in a dose-dependent manner. Our results suggest that VΔ2 cell function in the fetus is regulated by PD1 in order to limit inflammatory responses. Prenatal VΔ2 cell stimulation caused by maternal infection may induce long-term PD1 up-regulation, and hinder VΔ2 cell responses to pathogens and to BCG vaccination, affecting infant immunity.

1560

STATISTICAL MODELING METHODS REVEAL VARIABLE IMPORTANCE OF ANTI-MALARIAL ANTIBODIES IN KENYAN CHILDREN

Arlene E. Dent[1], Denise Babineau[1], Peter Sumba Odada[2], John Vulule[3], Ann Moormann[4], James Kazura[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]Kenya Medical Research Institute, Kisumu, Kenya, [3]Centers for Disease Control and Prevention, Kisumu, Kenya, [4]University of Massachusetts, Worcester, MA, United States

Naturally acquired immunity (NAI) to *Plasmodium falciparum* is characterized by age-related control of parasitemia and protection from clinical malaria. With the goal of advancing knowledge of how the magnitude and breadth of anti-malaria IgG antibodies contribute to NAI, we used plasma from 97 children (1-14 years) who participated in a treatment time to infection study in western Kenya. IgG antibodies to 24 recombinant merozoite and pre-erythrocytic proteins were measured by multiplex microsphere assay. A global test had a p value of 0.0601 indicating that there is evidence that antibodies against at least one of the antigens is associated with delayed time to infection. Traditionally we have used only Kaplan-Meier analysis to examine the relationship between antibody responses and time to infection. Here we developed and compared 6 prediction models: 1) Kaplan-Meier, 2) Univariate Screening, 3) Backward Elimination, 4) Penalized Regression Models--Least Absolute Shrinkage and Selection Operator (lasso) 5) Penalized Regression Model--Elastic net, and 6) Random Survival Forests. Each method has benefits and limitations. By comparing the results of all analyses and evaluating the performance of each using the time-dependent Brier score, several promising antigen targets that appear to contribute to protection from infection such as AMA1(FVO), MSP3, MSP1 (3D7), and EBA181 would have been missed if we only used Kaplan-Meier analysis. We conclude that high level statistical models offer insights into targets of NAI and identify potential candidates for inclusion in multi-antigen malaria vaccines.

1561

TRACKING THE DEVELOPMENT OF IMMUNITY TO *PLASMODIUM FALCIPARUM* AFTER IMMUNIZATION WITH IRRADIATED SPOROZOITES IN A MALARIA ENDEMIC SETTING

Irfan Zaidi[1], Hama Diallo[2], Yeya Dit S. Sarro[3], Bourahima Kone[3], Abdoulaye Katile[2], Amadou Kone[3], Ousmane Koita[3], Freda Omaswa[4], Sharon Wong-Madden[1], Mahamdou S. Sissoko[2], Stephen L. Hoffman[4], Ogobara Doumbo[2], Sara A. Healy[1], Michael Walther[1], Patrick Duffy[1]

[1]Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, Rockville, MD, United States, [2]Malaria Research and Training Center, Mali-NIAID ICER, University of Science, Techniques and Technologies of Bamako, Bamako, Mali, [3]SEREFO Core Immunology Laboratory, ICER, University of Science, Techniques and Technologies of Bamako, Bamako, Mali, [4]Sanaria Inc., Rockville, MD, United States

Vaccination with irradiated *Plasmodium falciparum* (Pf) sporozoites (SPZ) has been shown to induce sterilizing protection against malaria infection in naïve volunteers, but as yet this has not been tested in subjects living in malaria endemic regions who would have pre-existing immunity. In collaboration with Sanaria Inc. and the Malaria Research Training Centre (MRTC), the first double-blinded randomized phase 1b trial of the Sanaria®

PfSPZ Vaccine (radiation attenuated, aseptic, purified PfSPZ) in a malaria endemic region is being conducted in Mali. Ninety-three volunteers were randomized to receive five vaccinations of $2.7x10^5$ PfSPZ or normal saline placebo. In addition twelve volunteers received two vaccinations to ascertain safety of the vaccine, and nine of these volunteers will receive 5 immunizations. Vaccinations began in January 2014, and will be completed in July 2014. CD8 T cells may be the key mediators of protection against liver stages of *P. falciparum*, but other cellular subsets may also play a significant role. In earlier studies, CD38 and CD11a on CD8 T cells have been used to measure the development of immunity against malaria antigens after vaccination in mice and humans. Whole blood samples are being collected at baseline, 3, 7 and 27 days after each vaccination, and used to measure the percentages of CD8, CD4, T and NK cells expressing CD38 and CD11a using flow cytometry. The results after each of the 5 vaccine doses will be reported, and may be useful in discriminating the roles of various immune subsets in conferring protection after PfSPZ vaccination.

1562

ANTIBODY PROFILING BY PROTEIN MICROARRAY IN NAÏVE AND SEMI-IMMUNE INDIVIDUALS IN COLOMBIA AFTER EXPERIMENTAL CHALLENGE WITH *PLASMODIUM VIVAX*

Huw Davies[1], Myriam Arévalo-Herrera[2], Mary López-Pérez[2], Aarti Jain[1], Kelly Rubiano[2], José Millán Oñate[3], Philip L. Felgner[1], Socrates Herrera[2]

[1]University of California Irvine, Irvine, CA, United States, [2]Caucaseco Scientific Research Center, Cali, Colombia, [3]Centro Médico Imbanaco, Cali, Colombia

Acquisition of naturally acquired immunity to malaria in low transmission intensity regions is often accomplished from relatively few exposures and can occur at any age. This stands in contrast to high transmission areas where protection is acquired from repeated exposures during the first two decades of life. In this study we have evaluated serological differences in response to live *Plasmodium vivax* challenge in two groups of individuals from Colombia. These comprised 7 individuals with no history of malaria ("naïve"), and 9 individuals naturally exposed to *P. vivax* previously ("semi-immune"). Each individual was infected with *P. vivax* sporozoites via mosquito bites. As a result, 6 naïve (86%) and 6 semi-immune (67%) individuals developed clinical symptoms in the second week post-challenge. Fever and headache were significantly more frequent or severe in naïve compared to semi-immune individuals, while blood alanine aminotransferase, aspartate aminotransferase (markers of liver function) and C-reactive protein were also significantly higher in naïve. Overall, the clinical data indicated previous exposure to *P. vivax* is associated with protection against clinical symptoms in response to *P. vivax* challenge. To test whether protection might also be associated with IgG profiles, serum from d0, d5, d11, 3 weeks, and 4 months post-challenge were probed on a protein microarray displaying 500 *P. vivax* and 500 *P. falciparum* sero-reactive exon products. The array did not reveal strong serological differences between naïve and semi-immune individuals at the time of challenge. However, a difference was observed in the response to challenge with *P. vivax* sporozoites. In both groups, the response peaked at week 3 and declined thereafter, with the response by the naïve group being noticeably stronger and broader in comparison with the semi-immune group. Interestingly, the bulk of the serological response seen in the semi-immune group was also associated with those individuals with fever or headache, while those that were asymptomatic had an attenuated response. Thus the association between previous exposure and protection against clinical malaria is also associated with lower serological reactivity as measured by protein array, possibly reflecting activity of the memory pool in previously-exposed individuals.

1563

KIR3DS1 HOST GENOTYPE, IL17 SERUM CONCENTRATION AND *PLASMODIUM VIVAX* CSP GENOTYPES MODULATION OF VIVAX MALARIA PARASITEMIA

Giselle M. Viana[1], Andréa Luciana da Silva[2], Erian de Almeida Santos[3], Danielle Regina Barbosa[1], Gustavo Capatti Cassiano[4], Priscila Vianna[5], Nadine Glesse[5], José Artur Chies[5], Valéria Suenya Leal[2], Lilian de Araújo Mendes[2], Janaina Mota de Vasconcelos[3], Ricardo Luiz Machado[1], João Farias Guerreiro[2], Marinete Marins Póvoa[1], Eduardo José dos Santos[2]

[1]Instituto Evandro Chagas - IEC/SVS/MS, Ananindeua, Brazil, [2]Universidade Federal do Pará - UFPA, Belém, Brazil, [3]Universidade Federal do Pará - UFPA, Instituto de Ciências Biológicas, Belém, Brazil, [4]Faculdade de Medicina de São José do Rio Preto - FAMERP, São José do Rio Preto, Brazil, [5]Universidade Federal do Rio Grande do Sul - UFRGS, Porto Alegre, Brazil

Malaria is the most important arthropod borne disease in Brazil, occurring mainly in North region, being a serious Public Health concern, in terms of its high morbidity and mortality rates. Both host and parasite genetic features, as well as host immune profile, modulate resistance to infection and heterogeneity of clinical/laboratorial manifestations. This study approached: i) the *Plasmodium vivax* genotypes in Circumsporozoite Protein (CSP) region; ii) host KIR genes polymorphisms and; iii) immune profile during the infection (concentration of six circulating cytokines: IL-17, INF-g, TNF-a, IL-10, IL-6, IL-4, IL-2). Furthermore, we evaluated how these factors modulate the parasitemia and how host KIR polymorphism influences the number of CSP genotypes in infected individuals. Fourteen KIR genes and their ligands were genotyped by PCR-SSP on 62 *P. vivax*-infected individuals living in the town of Goianesia do Pará (Pará, Brazil). Cytokine levels were quantified using a Becton Dickinson cytometric bead array. CSP genotypes (Vk210, Vk247 and *P.vivax*-like) were determined by PCR-RFLP. Among the 14 KIR genes only KIR3DS1 presence was associated with higher parasitemia (Mann-Whitney test; p=0.01). Moreover, KIR3DS1 presence associates with *P. vivax* CSP multiple genotypes (Fisher Exact test; 0.0084). Interestingly, individuals presenting multiple genotypes of *P. vivax* CSP showed also higher parasitemia (Mann-Whitney test; p=0.028). Fulfilling this scenario IL-17 concentration correlated negatively with parasitemia (r=-0.6702; p=0.024), suggesting a protective role in the parasitemia control. Noteworthy, KIR3DS1 is a key stimulatory receptor of Natural Killer cells that produces many cytokines related to immune response to *P.vivax* infection being the results suggestive of a role of this gene in control of the number of different circulating CSP genotypes as well as parasitemia and highlights KIR3DS1 role's in malaria immune response in an Amazonian population.

1564

INDUCTION OF HOST CELL AUTOPHAGY PROMOTES THE DEVELOPMENT OF MALARIA LIVER STAGE

Wenyue Xu, Chenghao Zhao, Taiping Liu, Taoli Zhou

Third Military Medical University, Chongqing, China

Host cell autophagy has been reported to be involved in the restriction of the growth of a variety of microorganisms, but its role in the development of malaria pre-erythrocytic stage is still unknown. Here, we found that sporozoite infection induced LC3 containing vesicle to surround the exo-erythrocytic forms in hepatocyte, indicating the hepatocyte autophagy of the malaria parasite. Rapamycin treatment significantly increased the number of the autophagy of exo-erythrocytic form by hepatocyte, and promoted the fusion of autophagy containing malaria parasite with lysosome. However, host cell autophagy induced by rapamycin could significantly promote the development of exo-erythrocytic form *in vitro*. Further study showed that parasites inside the autophagosome could still survive and replicate normally as same as those in the parasitphorous vacuole, and the acidification of autolysosome was greatly inhibited. Therefore, we firstly provide evidence that sporozoite infection could induce host cell autophagy of malaria parasite, and the

induction of hepatocyte autophagy promoted the development of pre-erythrocytic stage, which might be associated with its ability to suppress the acidification of autolysosome. This data indicated the induction of autophagy as a novel escape strategy of exo-erythrocytic stage, and shed new light on the prophylatic therapy against liver stage.

1565

TRANSCRIPTOME ANALYSIS OF ATYPICAL MEMORY B CELLS IN THE SETTING OF NATURAL *PLASMODIUM FALCIPARUM* INFECTION

Richard T. Sullivan[1], Mary Fontana[1], Isaac Ssewanyana[2], Harriet Mayanja[3], Prasanna Jagannathan[1], Margaret Feeney[1], Charlie Kim[1], Bryan Greenhouse[1]

[1]University of California San Francisco, San Francsico, CA, United States, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]Makerere University Medical School, Kampala, Uganda

Higher frequencies of an "atypical" phenotype of memory B cells have been associated with chronic exposure to *Plasmodium falciparum* and other pathogens, but the function of these cells in malaria pathogenesis and the development of immunity is not understood. Atypical memory B cells have been hypothesized to be dysfunctional based on their poor production of antibodies *in vitro*, while others have hypothesized that these cells actively produce antibodies. However, antibody secretion is not the sole function of B cells. To better understand the functional nature of atypical memory B cells, we performed a systematic evaluation of the differences between atypical and classical memory B cells using transcriptome analysis of both subsets in the presence of naturally occurring *P. falciparum* infection. B cells subsets were isolated from 6 parasitemic but asymptomatic children aged 8-10 years old and analyzed on whole genome microarrays. Expression of select genes was confirmed by qPCR and/or flow cytometry. Consistent with previously hypothesized atypical memory B cell dysfunction, we found a number of inhibitory genes elevated compared to classical memory B cells. However, atypical memory B cells were not dormant, but clearly metabolically active. In addition to elevated inhibitory genes, atypical memory B cells also upregulated multiple genes associated with activation, migration, and secretion pathways. Upregulation of genes in these pathways suggests a more complex function for atypical memory B cells beyond antibody secretion. Indeed, we performed functional assays confirming that these cells did not spontaneously secrete IgG *ex vivo*. We are currently performing additional functional assays to help define other roles atypical memory B cells have in modulating cellular responses. In summary, the transcriptome data suggest a functional role for atypical B cells, which, while as yet unknown, may be independent of antibody production.

1566

AGE-SPECIFIC MALARIA SERO-CONVERSION RATES IN HAITI: AN ANALYSIS OF MALARIA TRANSMISSION IN THE OUEST AND SUD-EST DEPARTMENTS OF HAITI

Michael E. von Fricken[1], Brandon Lam[1], Thomas A. Weppelmann[1], Will Eaton[1], Laura Schick[2], Roseline Masse[3], Madsen V. Beau De Rochars[1], Benjamin Anderson[1], Bernard A. Okech[1]

[1]University of Florida, Gainesville, FL, United States, [2]Community Coalition for Haiti, Jacmel, Haiti, [3]Christianville Foundation, Inc., Gressier, Haiti

Malaria transmission continues to occur in Haiti, with 32,000 confirmed cases of *Plasmodium falciparum* reported in 2011. As rates of malaria decrease, passive surveillance measures become less sensitive for capturing transmission intensity. By implementing highly sensitive antibody detection methods we aimed to quantifying malaria transmission intensity over time. A total of 770 serum samples were screened for malaria antibodies using indirect enzyme-linked immunosorbant assay (ELISA) coated with vaccine candidates, apical membrane antigen (AMA-1) and merozoite surface protein-11-19 (MSP-1). The "exposed" cut off value was established based on three standard deviations above the normal distribution of our negative serum absorbances (OD of 0.37 and 0.48 for AMA-1 and MSP-1 respectively). Between February and May 2013, sample collection occurred at four different sites; a rural community, two schools and a clinic in the Ouest and Sud-Est departments of Haiti. Of the 770 samples screened, 170 (22.1%) had been exposed to malaria at one point in their life time. Age was highly associated with the likelihood of having been exposed (p-value <0.001). After adjusting for age, the sero-conversion rate calculations indicated that the annual malaria transmission in the Ouest and Sud-Est department is roughly 1.03%. This data suggests that despite the absence of sustained malaria control efforts in Haiti, transmission has remained relatively low over multiple decades. Our results are further supported by passive hospital based surveillance conducted by the Haiti Health Surveillance system, which found low country-wide transmission (<1%). These findings provide valuable information that can be used to make a case for the elimination of malaria on the Island of Hispaniola.

1567

TOWARDS FULL *PLASMODIUM FALCIPARUM* PROTEOME AND REACTOME ANTIBODY SCREENING ASSAYS USING REPRODUCIBLE HIGH-THROUGHPUT PROTEIN ARRAYS

Joseph J. Campo[1], Douglas M. Molina[1], Chris Hung[1], Arlo Z. Randall[1], Li Liang[2], Arti Jain[2], D. Huw Davies[2], Elizabeth Baum[2], Peter D. Crompton[3], Robert W. Sauerwein[4], Robert Seder[5], Stephen L. Hoffman[6], Denise L. Doolan[7], John J. Aponte[8], Carlota Dobano[8], Angela Yee[1], Xiaowu Liang[1], Philip L. Felgner[2]

[1]Antigen Discovery, Inc., Irvine, CA, United States, [2]University of California Irvine, Irvine, CA, United States, [3]Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [4]Department of Medical Microbiology, Nijmegen, Netherlands, [5]Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [6]Sanaria, Inc., Rockville, MD, United States, [7]QIMR Berghofer Medical Research Institute, Herston, Australia, [8]Barcelona Centre for International Health Research (CRESIB), Barcelona, Spain

High throughput (HT) technologies have rapidly advanced discovery of novel targets for disease diagnostic tests and vaccines and is especially useful for pathogens with large, complex genomes, such as *Plasmodium* spp. We have developed a full proteome antibody screening assay for *P. falciparum* proteins expressed via an HT *in vitro* transcription and translation (IVTT) system and printed onto nitrocellulose-coated microarray chips. Here, we demonstrate the reproducibility of this protein array platform, the design and reactogenicity of the full proteome array, and the development of a down-selected *P. falciparum* "reactome" array. Comparisons included variation in assay dates, sample position on microarray chips and print batches. Agreement between replicate protein array assays was good. Mean difference and limits of agreement, demonstrated graphically with Bland-Altman dotplots, was 0.03 +/- 0.6 normalized intensity (range -6 to 8 on log scale). Correlation between replicate measurements of antibody breadth and magnitude were 0.91 and 0.95, respectively. Classification of antigen reactivity showed high concordance (Cohen's kappa: 0.95, p<0.01). The final down-selection and fabrication of a 1,000-feature "Pf1000 reactome" chip is underway and expected completion is in summer of 2014. We are down-selecting based on a tiered antigen reactivity scoring algorithm using antibody responses from serum/plasma samples of highly exposed individuals from sub-Saharan Africa and Papua New Guinea and samples from experimental models of pre-erythrocytic immunity. Reactive antigens are cross-referenced for confirmation with previously published data from other regions, including South America and Southeast Asia. The latest generation of down-selected chips will be a useful tool for seroepidemiological studies and screening for antibody immune correlates of protection, and full proteome chips can be used for identifying novel targets of monoclonal therapeutic antibodies or potential vaccine target antigens.

1568

IMMUNE MECHANISMS OF CROSS-STAGE PROTECTION BY VACCINATION WITH A LATE LIVER STAGE-ARRESTING GENETICALLY ATTENUATED PARASITE

Brandon K. Sack, Gladys Keitany, Jessica Miller, Ruobing Wang, Ashley Vaughan, Stefan Kappe

Seattle Biomedical Research Institute, Seattle, WA, United States

Immunizations targeting the pre-erythrocytic stages of *Plasmodium* have demonstrated sterile protection against malaria by blocking the sporozoite stages and/or eliminating liver stage parasites. Strategies such as irradiated sporozoite immunizations however face a formidable challenge in that they only confer stage-specific protection, and in consequence, less than complete protection can result in full-blown blood stage infection. However, we have demonstrated that immunization with sporozoites of *P. yoelii* genetically attenuated parasites (GAP), which arrest late in the liver stage development and do not progress to blood stage infection, engenders protective cross-stage immunity against a direct lethal blood stage challenge. Here, we demonstrate that immune mechanisms conferring cross-stage immunity are diverse. In C57BL/6 mice, antibodies are both sufficient and necessary for this protection as GAP-immunized mice depleted of T cells completely control blood stage parasitemia whereas immunized mice lacking antibodies succumb to uncontrolled blood stage infection. Conversely, BALB/c mice depend on T cells for cross-stage protection. C57BL/6 antibodies recognize antigens in late liver stages as well as on the surface of blood-stage merozoites but are not specific for the well-characterized merozoite surface protein (MSP)-1. In contrast, immunization of BALB/c mice engenders anti-blood stage antibodies but they are lower in quantity and do not recognize the surface of merozoites. Cross-stage protection is unique to late-liver stage arresting GAP as animals immunized with an early liver stage-arresting GAP are not protected from a lethal BS challenge and fail to generate antibodies which recognize late-liver stage/blood stage antigens. Therefore, immunization with a late liver stage-arresting GAP induces T and B cell responses capable of protecting against multiple stages of *Plasmodium* infection and thus constitutes the most potent among vaccination strategies. This unique system also offers an opportunity to identify novel protective antigens, which are shared with between both the liver stage and blood stage parasites.

1569

PERIPHERAL BLOOD FOXP3+ REGULATORY CD4 T CELLS DECLINE WITH INCREASING MALARIA EPISODES IN YOUNG CHILDREN

Michelle Boyle[1], Samuel Wamala[2], Prasanna Jagannathan[1], Iejoma Eccles-James[1], Katherine Bowen[1], Tara McIntyre[1], Lila Farrington[1], Felistas Nankya[2], Charles Ebusu[2], Mayimuna Nalubega[2], Kate Naluwu[2], Esther Sikomyu[2], Victor Bigira[2], James Kapisi[2], Jordan Tappero[3], Mary Kakuru[2], Emmanuel Arinaitwe[2], Charlie Kim[1], Grant Dorsey[1], Moses Kamya[2], Margaret Feeney[1]

[1]University of California San Francisco, San Francisco, CA, United States, [2]Infectious Diseases Research Collaboration, Kampala, Uganda, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States

Plasmodium falciparum infection has been reported to induce immunoregulatory cell populations such as FoxP3+ CD4 regulatory T cells (T_{regs}). Studies of malaria-naïve adults and low exposure regions have indicated that T_{regs} expand during acute malaria infection and can be induced by malaria *in vitro*, however in children the relationship is less clear. T_{reg} expansion may be associated with the slow acquisition of immunity seen in children from malaria endemic areas. Indeed, other chronic diseases have been shown to induce T_{regs} which contributes to ongoing infection. We investigated the frequency and function of T_{regs} in well characterized cohorts of two-year-old (n=79) and four-year-old (n=72) children in Tororo, Uganda. All prior malaria episodes from age 6 months were documented and children were followed for 1 year following sampling. In both 2 and 4 year old children, we found that higher prior malaria incidence was strongly associated with lower frequencies of T_{regs} (2 yo cohort rho = -0.28, p=0.011; 4yo cohort rho = -0.35, p=0.005) However, there was no difference in frequencies of T_{regs} seen in children with or without current or recent parasitemia. Functional differences between T_{regs} from children with high or low prior incidences were investigated in *ex vivo* microarray analysis, specific and global suppression assays, and during the time course of acute infection. Our data suggests that repeated infection results in a loss of functionally suppressive T_{regs} from the peripheral blood. These data may indicate that although T_{regs} are induced following malaria infection in naïve individuals, this process becomes blunted after chronic repeated malaria exposure, which may have implications for the development of effective immune responses.

1570

INTERLEUKIN 8 AND TRANSFORMING GROWTH FACTOR-BETA (TGFB) AMONG MALARIA PATIENTS IN LAGOS, NIGERIA

Wellington A. Oyibo, Uche Igbasi, Ikechuckwu A. Ofili

College of Medicine of the University of Lagos, Nigeria, Lagos, Nigeria

The interaction between pro- and anti-inflammatory cytokines such as interleukin-8 (IL-8) and transforming growth factor beta (TGF-β) plays an important role in malaria pathogenesis and outcome.TGFβ, produced by a wide range of cells, has a pivotal role in the control of the transition between pro-inflammatory (Th1-type) and anti-inflammatory (Th2-type) response during the acute and resolving phases of malaria infection. The role of IL-8 in *Plasmodium falciparum* malaria is unknown although studies indicate it's likely use as a biomarker of intensity of malaria.The aim of this study was to measure the plasma levels of IL-8 and TGF- β in 136 individuals with malaria and correlate the production of these cytokines with the severity of the disease. IL-8 and TGF- βlevels were determined using enzyme-linked immunosorbent assay. The severity of malaria was established by parasitemia, clinical symptoms and haematological parameters. The level of IL-8 was found to be substantially elevated (508.8±755.1pg/ml) in malaria infected individuals and its value was significant in parasitemia levels (43200.0, $p<0.05$). In contrast TGF-β levels were found to be lower in malaria patients (23,672±30,703.8pg/ml) compared to non-malaria patients, the mean difference in levels of IL-8 between malaria positive and malaria negative individuals was statistically significant ($p<0.05$).The relationship between TGF-β levels and packed cell volume was negatively correlated ($r = -0.27$) . These findings suggest that fine mechanisms regulate the interaction between TGF-β andIL-8 in the immune response to *Plasmodium falciparum* infection, seemingly directing*in vivo* modulations in red cell population, and indicating a likely role in susceptibility to malaria

1571

IMPACT OF ACUTE MALARIA ON PRE-EXISTING ANTIBODY LEVELS TO COMMON CHILDHOOD VACCINES

Jill Coursen[1], Silvia Portugal[1], Aissata Ongoiba[2], Boubacar Traore[2], John T. Harty[3], Peter D. Crompton[1]

[1]National Institutes of Health, Rockville, MD, United States, [2]University of Sciences, Technique and Technology of Bamako, Bamako, Mali, [3]University of Iowa, Iowa City, IA, United States

Vaccine-induced protective immunity against many pathogens relies on long-lived plasma cells that maintain basal levels of antibody. Recent evidence from animal models suggests that *Plasmodium* infection mediates a transient drop in antibody titers induced by prior influenza virus infection, raising the possible public health concern that malaria may be detrimental to previously generated vaccine responses. Prior studies in humans have assessed the impact of concurrent *P. falciparum* infection on responses to standard childhood vaccines, but to our knowledge the impact of acute *P. falciparum* infection on previously induced vaccine responses is unknown. To address this question we conducted a

longitudinal analysis of IgG titers specific for common viral (measles, polio, Hepatitis B) and bacterial (*Haemophilus influenzae type b*, meningococcus, tetanus) vaccine antigens in 54 children living in an area of Mali where the 6-month malaria and dry seasons are sharply demarcated. Vaccine-specific IgG titers were measured for each subject at five time points over an 18 month period: before and after the first dry season, during and 10 days after the first episode of febrile malaria of the ensuing malaria season, and at the end of the second dry season. Preliminary analyses suggest that average IgG decay rates are not significantly accelerated by acute *P. falciparum* infection; however, at the individual level a minority of children exhibited accelerated IgG decay rates following *P. falciparum* infection compared to decay rates over the dry season. In addition, preliminary comparisons with studies of non-malaria exposed populations suggest that overall decay rates of vaccine-induced IgG responses may be higher in the population of children who experienced febrile malaria. These data highlight the need for additional studies to understand the factors underlying variability in vaccine-specific antibody decay rates in individuals residing in malaria endemic areas.

1572

THE IMPACT OF *SCHISTOSOMA HAEMATOBIUM* INFECTION ON *PLASMODIUM FALCIPARUM*-INDUCED IMMUNE RESPONSES

Safiatou N. Doumbo[1], Jules Sangala[1], Jacqueline Moebius[2], Silvia Portugal[2], Tuan M. Tran[2], Aboudramane Bathily[1], Aissata Ongoiba[1], Peter D. Crompton[2], Boubacar Traore[1], Ogobara K. Doumbo[1]

[1]University of Sciences, Technique and Technology of Bamako, Bamako, Mali, [2]National Institutes of Health, Rockville, MD, United States

Plasmodium falciparum and *Schistosoma haematobium* often overlap in tropical and subtropical countries and impose tremendous disease burdens. Evidence suggests that *S. haematobium* modifies the risk of febrile malaria; however, the nature of this putative immunomodulation remains unclear. To investigate this question we analyzed *S. haematobium*-infected individuals (n=15) and uninfected controls (n=24) within a longitudinal cohort study in Mali. Before the malaria season, peripheral blood mononuclear cells (PBMCs) were collected from *S. haematobium*-infected and uninfected individuals, all of whom were negative for *P. falciparum* by PCR. PBMCs were analyzed before and after *in vitro* stimulation with lysate of *P. falciparum* (3D7) infected red blood cells (iRBCs) and analyzed by flow cytometry with intracellular cytokine staining; and supernatants of stimulated PBMCs were analyzed by a multiplex cytokine assay. Compared to uninfected controls, *S. haematobium*-infected individuals had a higher baseline percentage of dendritic cells, but no differences were observed in the proportion of B cells, T cells, NK cells or their respective subsets. Stimulation with iRBCs showed a modest increase in IFN-γ producing $CD4^+$ T cells in *S. haematobium*-infected individuals. These preliminary data suggest that *S. haematobium* modulates the innate and adaptive immune response to *P. falciparum* infection. In an ongoing longitudinal study we are studying the impact of concurrent or recent *S. haematobium* infection on the host immune response to subsequent *P. falciparum* infection.

1573

AVIDITY OF NATURALLY ACQUIRED ANTI-*PLASMODIUM VIVAX* MSP1-19 ANTIBODIES IN INDIVIDUALS PRESENTING DIFFERENT CLINICAL EXPRESSIONS OF MALARIA

Maria Carmen A. Sanchez, Adeiana G. Lima, Arianni R. Sanchez, Eduardo M. Ramos-Sanchez, Silvia M. Di Santi, Marina P. Dornelas, Sandra L. Moraes, Hiro Goto

São Paulo University, São Paulo, Brazil

Plasmodium vivax is the most prevalent species in Brazil accounting for around 85% of clinical cases. *P. vivax* infections cause non-severe malaria in most cases, but may also be asymptomatic or cause severe disease. C-terminal region of merozoite surface protein 1 of *P. vivax* (PvMSP1-19) is highly immunogenic conserved region that plays a major role in the protective immunity against asexual blood stages of malaria parasites. Since this protective immunity has been shown to correlate with levels of anti-MSP1-19 antibodies, this study aimed to evaluate the humoral immune response against PvMSP1-19 of individuals naturally exposed to malaria, from endemic areas of Brazil, in order to assess the IgG and IgM profile, the avidity of IgG antibody (functional affinity) and their association with different malaria clinical presentations. Serum samples from four groups of individuals were studied: severe malaria (N=18), asymptomatic infection (N=17), non-severe symptomatic malaria undergoing their first malaria episode (N=104) and non-severe symptomatic malaria with previous malaria episodes (N=102). All were positive for *P. vivax* by thick blood smear and/or PCR and for IgG and/or IgM antibodies by ELISA-PvMSP1-19. High avidity (>50%) IgG antibodies were observed in 92.9% of patients who had previous malaria episodes (median reactivity index: IgG=8.7 and IgM=1.1) and in 88.0% of asymptomatic individuals (median reactivity index: IgG=2.4 and IgM=0.3). Low/moderate avidity (≤50%) was seen in 89.1% of patients undergoing their first malaria episode (median reactivity index: IgG=6.7 and IgM=3.0) and in 94.0% of severe malaria patients (median reactivity index: IgG=7.7 and IgM=6.7). Predominance of high-avidity antibody in individuals with non-severe malaria that had multiple episodes of malaria and in asymptomatic infections corroborates the protective role of humoral immunity. It likely reduces the risk to develop mild and severe malaria. Our results show that protective immunity not only correlate with levels of anti-MSP1-19 antibodies but also with the quality of these antibodies.

1574

GENERATION OF "FULLY HUMAN" MONOCLONAL ANTIBODIES AGAINST THE CIRCUMSPOROZOITE PROTEIN (CSP) OF *PLASMODIUM FALCIPARUM* USING HUMANIZED HLA MICE

Sai P. Majji[1], Wathsala Wijayalath[1], Soumya Shashikumar[1], Sandra Inoue[1], Andrea Akyeampong[1], Thomas L. Richie[1], Eileen Villasante[1], Teodor-Doru Brumeanu[2], Sofia Casares[1]

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

"Fully human" monoclonal antibodies (hmAb) are a novel approach to treat infectious diseases. Fully human mAbs are devoid of the complications associated to the use of mAbs derived from mouse or humanized antibodies. We have generated humanized HLA mice in NOD.RagKO.IL2RgcKO background that develop a functional human immune system and respond to vaccination. Using human B cells from humanized HLA mice immunized with irradiation-attenuated *Plasmodium falciparum* sporozoites we have generated a panel of hmAbs against *P. falciparum* CSP. Herein we present data on anti-CSP hmAb immunocharacterization and *in vitro* and *in vivo* anti-parasitic activity.

1575

MONITORING AND EVALUATION OF EFFECTIVENESS OF IFAKARA HEALTH AND DEMOGRAPHIC SURVEILLANCE SYSTEMS (HDSS) IN QUANTIFYING IMPACT OF MALARIA CONTROL STRATEGIES AND INTERVENTIONS

Mwajabu M. Mwanaupanga

Ifakara Health Institute, Dar es salaam, United Republic of Tanzania

Health and Demographic Surveillance Systems (HDSS) have been set up in various sites in Africa and Asia. In the absence of effective vital registration and information on mortality, these health and demographic surveillance systems have well developed structures and standard operating procedures established to periodically monitor vital events like births, deaths and migrations in well defined areas to produce population health information. In Tanzania Ifakara DSS remain as an instrumental infrastructure of

producing key demographic indicators that are useful for planning and resource allocation to the community. The aim of my study is to determine the effectiveness of Ifakara HDSS in quantifying the impact of malaria control strategies and interventions. A cross sectional study design will be used to assess HDSS documentation mechanism on the impact of different malaria control strategies and interventions performed in the area, and to determine if the project effectively quantifies the impact of those interventions and strategies. Systematic sampling will be used to obtain number and types of documents that will be reviewed to fulfill the aim of our study. Collection and analysis of data will be both qualitative and quantities approach. Findings will be explained based on: 1) extent the knowledge and skills of the project staff in content of the HDSS malaria forms and data quality checking has increased from the pre to the post intervention periods of the project, 2) extent to which the malaria information management has improved, 3) ways in which the project was able to promote quality malaria data generation, 4) extents to which costs were able to reach project goal of documenting impact of malaria control strategies and interventions, and 4) how the HDSS understood and cared about the importance of resources at work and if the managers supported them with training, supervision and needed resources. The conclusion will be based upon findings that will be obtained during the data collection.

1576

BUILDING A CRITICAL MASS OF HEALTH WORKFORCE TO FIGHT MALARIA IN NIGERIA

Muhammad K. Salihu[1], Bolatito Aiyenigba[1], Veronica Momoh[2], Abba Z. Umar[1]

[1]FHI360, Abuja, Nigeria, [2]Health Partners International, Abuja, Nigeria

The Presidential Malaria Initiative (PMI) through the Malaria Action Program for States (MAPS) in Nigeria currently supports seven states to implement effective malaria control, prevention, diagnosis and treatment activities. Nigeria health workforces are diverse and cut across administrative, programmatic and clinical areas including community health services. Effective and sustainable malaria program will need to build capacity in all these areas. This study documents the PMI achievement in Nigeria. In-service training using adult learning principles were conducted for all cadres of staff relevant to malaria prevention and control. Administrative and programmatic staffs were trained on malaria program management; clinical staff were trained on prevention of malaria in pregnancy (MIP); malaria diagnosis using rapid test and microscopy; malaria case management including severe malaria. Other trainings conducted were focused on strengthening health information management; behavior change communication and community acceptance of interventions. From October 2011 to September 2013, 2770 health workers had been trained on MIP; over 9400 health workers and Community Care Givers had been trained on case management and 3380 trained on malaria diagnosis. About 2498 health managers were also trained on malaria program management. Over 4504 health workers were trained on health information management; 3344 health educators and journalists trained on malaria BCC. Programmatic results recorded include increase in fever cases tested from 45.9% in April 2013 to 70.2% in March 2014; and referral of over 40,000 pregnant women and children under 5 for ANC and treatment of fever. The critical mass of health workers trained will contribute to the implementation of a sustainable, efficient, integrated malaria program at state and local government levels because many of these workers offer informal services in their communities. Pre-service training is needed to sustain malaria control program.

1577

QUALITY OF CARE FOR CHILDREN UNDER FIVE YEARS WITH UNCOMPLICATED MALARIA AT PRIVATE CLINICS IN MAKINDYE DIVISION, KAMPALA DISTRICT

Ronald Mulebeke[1], Achillies Katamba[2], Aggrey Mukose[1], Pauline Byakika[2], Ellizeus Rutebemberwa[1], Phyllis Awor[1], Jane Achan[2], Adoke Yeka[1]

[1]Makerere University School of Public Health, Kampala, Uganda, [2]Makerere University College of Health Sciences, Kampala, Uganda

Over 60% of the population in developing countries seeks for care from private health facilities. In Uganda over 80% of the Population access health care from small drug shops, private clinics and private-not-for profit providers because they are closer to the communities and are perceived to be affordable. However, health providers in private clinics more frequently violate accepted medical standards and guideline because there primary goal is making profit. Quality of care for children with uncomplicated malaria in such settings is generally sub-optimal with low adherence to treatment guidelines. We are conducting a study to assess the quality of care provided to children under five years with uncomplicated malaria at private clinics in Makindye division, Kampala district, Uganda. The study is a cross-sectional cluster survey conducted in 30 private clinics. Data was collected using patient exit interviews, questionnaires for health workers, observation of health workers during consultation, health facility audits and Key Informant Interviews. A total of 180 exit interviews were conducted with caretakers of children and 30 healthcare workers were observed while treating patients and were interviewed thereafter. A total of 8 Key informant interviews were conducted with heads of private clinics. Data is being analyzed to determine predictors of quality of care for children with uncomplicated malaria at private clinics. A composite index will be utilized to assess overall quality of care.

1578

FACTORS AFFECTING TREATMENT SEEKING FOR FEVER BEFORE AND AFTER INTERVENTIONS TO IMPROVE ACCESS AND TARGETING OF ARTEMISININ COMBINATION THERAPIES

Charles Festo[1], Rebecca Thomson[2], Boniface Johanes[1], Admirabilis Kalolella[1], Happy Nchimbi[1], Katia Bruxvoort[2], Matthew Cairns[2], Mark Taylor[2], Immo Kleinschmidt[2], S. Patrick Kachur[3], Catherine Goodman[2]

[1]Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States

Artemisinin combination therapies (ACTs) were introduced as the first line antimalarial in Tanzania in 2006, but access and targeting were poor. The main sources of treatment for fever included public and private health facilities, drug stores, pharmacies and general shops, but the probability of receiving both parasitological diagnosis and an ACT varied considerably across these outlet types. It is therefore essential to understand factors that determine choice of provider. We assessed this using data from large scale household surveys conducted before (2010) and after (2012) national rollout of rapid diagnostic tests in public facilities, and ACT subsidies under the Affordable Medicines Facility-malaria. We visited a representative random sample of households in each of 3 regions (Mwanza, Mbeya, Mtwara) with varying malaria transmission and access to health care. 5,423 households at baseline and 5,511 at endline were sampled using a multi-stage design. All household members reporting fever were asked about treatment sought. There was no significant change in the percentage of people with fever seeking care between baseline and endline (69.5% and 73.6%, p=0.07). However, there were changes in treatment source, with an increase in the percentage using drug stores (41.3% to 54.1%, p<0.001), and a fall in use of public facilities (25.3% to 16.8% p<0.001). Overall, children <5 years old were more likely to be taken to a public facility at baseline and endline. We will present results

of multivariable analysis assessing the adjusted odds of seeking care, and of seeking care at specific provider types at baseline and endline. Relevant exposures include age, sex, region, urban/rural location, education of the household head, time to the nearest treatment source, enrolment in health insurance scheme and local ACT stockout levels in public facilities. Key factors affecting treatment seeking decisions will be discussed, including how these have changed following implementation of two key interventions affecting malaria treatment provision, and the implications for future policy to enhance access and targeting of ACTs.

1579

AN ASSESSMENT OF THE MALARIA-RELATED KNOWLEDGE AND PRACTICES OF TANZANIA'S DRUG RETAILERS: EXPLORING THE IMPACT OF DRUG STORE ACCREDITATION

Rebecca Thomson[1], Boniface Johanes[2], Charles Festo[2], Admirabilis Kalolella[2], Mark Taylor[1], Sarah Tougher[1], Yazoume Ye[3], Andrea Mann[1], Ruilin Ren[3], Katia Bruxvoort[1], Barbara Willey[1], Fred Arnold[3], Kara Hanson[1], Catherine Goodman[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania, [3]ICF International, Calverton, MD, United States

Since 2005 Tanzania has been upgrading its approximately 7,000 drug stores to Accredited Drug Dispensing Outlets (ADDOs), involving dispenser training, introduction of record keeping and enhanced regulation. ADDOs are permitted to stock 49 prescription-only medicines, including artemisinin combination therapies. Prior to accreditation drug stores could officially stock over-the-counter medicines only, though many stocked prescription-only antimalarials. Oral artemisinin monotherapies and injectables were not allowed in any drug stores. By late 2011 ADDO conversion was complete in 14 of 21 regions. We explored variation in malaria-related knowledge and practices of drug retailers in ADDO and non-ADDO regions. We excluded Dar es Salaam where market conditions were not comparable to other regions. Data were collected as part of the Affordable Medicines Facility-malaria Independent Evaluation, involving a nationally representative survey of antimalarial retailer in October-December 2011. We randomly selected 49 wards, and interviewed all drug stores stocking antimalarials. Interviews were conducted in 148 drug stores in ADDO regions and 127 in non-ADDO regions. Drug stores in ADDO and non-ADDO regions were similar in terms of employing staff with health-related qualifications (96.1% and 96.2%, p=0.99); stocking the first line antimalarial (59.5% and 60.7%, p=0.89); and stocking artemisinin monotherapy (0.9% and 0.0%, p=0.43). Drug stores in ADDO regions performed better on knowledge of the first line antimalarial (99.5% and 91.5%, p=0.001). There was weak evidence of a lower price and higher market share of the first line antimalarial in ADDO regions. However, drug stores in non-ADDO regions were less likely to stock injectables (21.5% and 3.6%, p=0.003). ADDO conversion is frequently cited as a model for improving retail sector drug provision. Drug stores in ADDO performed better on some but not all indicators, possibly indicating weaknesses in ADDO regulation and high staff turnover. More evidence is needed on the value-added and value for money of ADDO roll out to inform retail policy in Tanzania and elsewhere.

1580

AUDITING VILLAGE HEALTH TEAMS' CAPACITY FOR MANAGEMENT OF MALARIA: RESULTS OF THE 2013 ACTWATCH UGANDA OUTLET SURVEY

Julius Njogu[1], John H. Rogers[2], Henry Kaula[3], Vamsi Vasireddy[4], Megan Littrell[5]

[1]Population Services International, Nairobi, Kenya, [2]Centers for Disease Control and Prevention, Dar es salaam, United Republic of Tanzania, [3]PACE, Kampala, Uganda, [4]Independent Consultant, Chicago, IL, United States, [5]Population Services International, Washington, DC, United States

Malaria is endemic across 95% of Uganda. The Ugandan government aims at 85% of malaria cases receiving prompt and recommended treatment by the end of 2014. To achieve this goal, integrated Community Case Management (iCCM) initiatives have been implemented in 34 districts by building capacity of Village Health Teams (VHTs) to manage febrile children. Within these initiatives, all suspected malaria cases are tested and confirmed cases are treated with Artemether-Lumefantrine (AL), and severe cases are referred for rectal Artesunate treatment. To enumerate VHT malaria capacity under iCCM, a census of VHTs was conducted in 16 iCCM sub-counties using ACTwatch methodology. A questionnaire was administered to assess knowledge, availability of antimalarials and rapid diagnostic tests (RDTs). We present descriptive results on availability and knowledge. We surveyed a total of 1,862 VHTs. Of these, 33.7% (628) stocked AL, 10.0% (187) rectal artesunate and 21.6% (403) stocked malaria RDTs during the survey visit. Notably, when AL was available, 56.5% (355/628) of VHTs stocked malaria RDTs. Of those with antimalarials, 91.6% (606/660) correctly stated recommended first-line medicine for uncomplicated malaria (AL) and 83.3% knew its dosing regimen. Among RDT stockists, 93% (375/403) stated they would never dispense antimalarial following a negative RDT result. The iCCM presents an important channel for increasing access to integrated case-management in Uganda. According to these results, VHTs are highly knowledgeable but lack antimalarial and RDT stocks, which may undermine iCCM goals. Enhancing stable and reliable supply of first-line medicines and RDTs may sustain increased access to prompt and correct malaria-case management.

1581

ASSESSING THE COST OF PRIVATE SECTOR ACT SUBSIDIES - THE FINANCIAL AND ECONOMIC COSTS OF THE AFFORDABLE MEDICINES FACILITY - MALARIA (AMFM) IN THREE AFRICAN COUNTRIES

Catherine Goodman[1], Fiona Cianci[1], Daniel Cobos[2], Sarah Tougher[1], Kara Hanson[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Swiss Tropical and Public Health Institute, Basel, Switzerland

AMFm was designed to improve uptake of quality-assured artemisinin combination therapies (ACTs). Hosted by the Global Fund to Fight HIV/AIDS, Tuberculosis and Malaria, it operated in eight national scale pilots, involving: (i) price negotiations with ACT manufacturers; (ii) a subsidy for ACTs at the top of the global supply chain; and (iii) supporting interventions, such as communications campaigns and provider training. An Independent Evaluation found that in most but not all settings AMFm improved the availability, affordability and market share of quality-assured ACTs. However, no data were available on AMFm costs. This study aimed to address this gap. We collected data on the costs of implementing AMFm in the private for-profit sector only in Nigeria, Kenya and Madagascar, representing a range of performance on AMFm indicators. Costs were included for all AMFm-related activities at both country level and the Global Fund headquarters. We adopted a "funders perspective", covering all resources contributed by external funding agencies, NGOs and national governments, but excluding costs to commercial actors and households. Results are presented in terms of financial costs (actual expenditure) and economic costs (which include an annualised component

of start-up costs based on their expected useful life). All costs were converted to 2012 USD. The number of subsidised ACTs delivered for the private for-profit sector by the end of 2012 was 91,4 million (mn) in Nigeria and 28,3mn in Kenya, but only 2,1mn in Madagascar, where imports were much lower reflecting the very limited communications campaign, the predominance in the market of outlets not permitted to sell ACT, and political and economic disorder. Total financial costs for 2009-12 were $110,2mn in Nigeria, $39,9mn in Kenya and $4,9mn in Madagascar. Annual economic costs for 2011 were $57,2mn in Nigeria, $17,7mn in Kenya and $2,2mn in Madagascar, implying respectively an economic cost per capita of $0.35, $0.42 and $0.10, and per ACT dose delivered of $1.30, $1.51 and $1.91. The ACT subsidy itself accounted for 89% of economic costs in Nigeria, 80% in Kenya, and 48% in Madagascar. Sensitivity analysis will be presented to explore the impact of varying key assumptions and to estimate the costs of replication in other settings. The implications for the potential value for money of strategies to expand ACT use through the private sector will be discussed.

1582

INVESTIGATING THE DETERMINANTS OF DEMAND FOR ANTIMALARIAL MEDICINES IN BENIN, NIGERIA, UGANDA AND ZAMBIA

Benjamin Palafox[1], Kara Hanson[1], Catherine Goodman[1], Aurélia Lépine[1], Virginia Wiseman[1], Marcos Vera Hernandez[2]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]University College London, London, United Kingdom

Artemisinin-based combination therapy (ACT) is an essential health systems input in malaria endemic countries. By 2008, all of these countries had adopted ACT as the recommended first-line treatment for falciparum malaria. Although ACT may be available free of charge through public facilities, use of non-ACTs is common. This is particularly true for children under 5 years of age who may be treated presumptively where diagnostic capacity is limited. Much of these non-ACTs are purchased easily from private sector retailers who may also sell ACTs, but at much higher prices. Therefore, ensuring that children receive appropriate and quality treatment for malaria requires an understanding of how responsive household demand is to antimalarial prices and other determinants. We estimated econometric demand models for antimalarials to examine the determinants of antimalarial choice in Benin, Nigeria, Uganda and Zambia. In each country, data were collected through nationally representative surveys of households that experienced a recent paediatric febrile episode. This was complemented by survey data from all possible public and private sources of antimalarial medicines in the vicinity of these households. Treatment choices included ACTs, oral artemisinin monotherapies, non-artemisinin therapies, and no treatment. The range of determinants studied included various treatment price components (e.g. antimalarials, diagnostics, travel), and characteristics of the provider, household and caregiver. Our findings will focus on the most significant determinants of which antimalarial households obtain and from where, and examine how responsive antimalarial demand is to changes in antimalarial prices and household income. Given the considerable resources directed toward improving access to appropriate malaria treatment, we will also discuss how these findings may be applied to optimise the equitable impact of these investments in pluralistic health systems settings.

1583

DETERMINANTS OF STOCKING AND PRICING OF SUBSIDIZED ANTIMALARIAL TREATMENTS BY RETAILERS IN THE PRIVATE FOR-PROFIT SECTOR: EVIDENCE FROM NATIONAL SUBSIDY PROGRAMS IN KENYA, NIGERIA AND UGANDA

Sarah Tougher, Catherine Goodman, Kara Hanson

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

There is increased interest in improving quality of care in the private for-profit sector in low and middle income countries, as private providers are an important source of treatment for many illnesses. In the case of malaria, a large proportion of patients purchase antimalarial treatments from for-profit providers. For example, data from 2009-2010 show that in Nigeria, Kenya and Uganda, 97%, 67% and 40% of total antimalarial volumes were distributed by for-profit providers, respectively. However, medicines obtained are often inappropriate, because non-recommended treatments are widely and cheaply available. Subsidies for artemisinin combination therapies (ACTs), the first-line treatment in most malaria-endemic countries, have been implemented in a number of settings in order to improve coverage of ACTs and discourage use of other treatments. The largest initiative of this type was the Affordable Medicines Facility - malaria (AMFm), which was implemented at a national scale in eight pilots from 2010-2013. By the end of 2013, over 310 million treatments were ordered for the private for-profit sector through the initiative, and an Independent Evaluation reported large improvements in ACT availability, price and market share in six of the eight pilots. However, little is known about the causes of inter- and intra- country variations in performance. The success of private sector ACT subsidy programmes is determined by providers' decisions on whether to stock subsidized medicines and decisions on the pricing of subsidized medicines. This study used nationally-representative outlet-survey data from Kenya, Nigeria and Uganda to model provider decisions to stock ACTs subsidized through AMFm, and set markups for the drugs. These three countries were selected, because they have diverse contexts and AMFm had differing effects. For each country, multiple regression analysis was used to examine the determinants of markups and stocking of subsidized ACTs. The determinants investigated were a set of product, provider and market characteristics, including measures of competition. The analysis addressed the endogenous and hierarchical nature of the data. The evidence presented will help identify settings suitable for ACT subsidies and the types of supporting interventions and their targeting that are most appropriate.

1584

OPTIMAL PRICE SUBSIDIES FOR APPROPRIATE MALARIA TESTING AND TREATMENT BEHAVIOR

Kristian S. Hansen[1], Tine H. Lesner[2], Lars P. Østerdal[2]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]University of Southern Denmark, Odense, Denmark

Malaria continues to be a serious public health problem particularly in Africa. Limited access to antimalarials and over-treatment with antimalarials are two seemingly contradicting phenomena that co-exist. A global subsidy on selected antimalarial drugs has been suggested to increase access to the most effective treatment both in the public and private sectors. In order to also reduce over-treatment we propose a combined price subsidy on malaria rapid diagnostic tests and antimalarial drugs. Focusing on the private sector, we analyse the optimal subsidy combination that incentivises individuals suspecting themselves to have malaria to purchase a parasitological test before buying the recommended treatment using an expected utility model describing the health-seeking behaviour of a representative individual. Solving our model numerically for individuals with a range of different health-seeking behaviours shows that the optimal policy of the health planner is to redirect some of the subsidy money from antimalarial drugs to parasitological tests.

1585

IDENTIFYING MINIMAL EPITOPES ON THE SURFACE OF *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN REACTIVE WITH NEUTRALIZING MONOCLONAL ANTIBODIES

Miriam T. George[1], Jesse L. Schloegel[2], Francis B. Ntumngia[1], Samantha J. Barnes[1], Edwin Chen[3], Christopher L. King[4], Niraj H. Tolia[3], Joanne L. Casey[2], Michael Foley[2], John H. Adams[1]

[1]University of South Florida, Tampa, FL, United States, [2]La Trobe University, Victoria, Australia, [3]Washington University School of Medicine, St. Louis, MO, United States, [4]Case Western Reserve University, Cleveland, OH, United States

Plasmodium vivax Duffy Binding protein region II (PvDBPII) is an essential ligand for reticulocyte invasion, thereby making this molecule an attractive vaccine candidate to protect against asexual blood-stage *P. vivax*. Similar to other blood-stage vaccine candidates, DBP allelic variation elicits a strain-specific immunity that may be a major challenge for development of a broadly effective vaccine against vivax malaria. This study aims to identify conserved epitopes of neutralizing anti-DBP monoclonal antibodies (mAbs) using immunochemical and structural approaches to help design a strain-transcending vaccine. The crystal structure of PvDBPII consists of 2 α-helical bundles with an antiparallel β-hairpin near the N-terminus and may be assigned into three subdomains delineated by six disulphide bonds. The various subdomains and combination of subdomains were expressed in their correctly refolded and disulphide bonded conformation on the surface of the M13 filamentous phage. Additionally, a PvDBPII gene fragment library was used for biopanning to screen phage clones reactive with anti-DBP mAbs. Comparative analysis of specific targets of non-inhibitory anti-DBP mAbs with neutralizing anti-DBP mAbs will help determine essential regions of PvDBP for a subunit vaccine designed to protect against blood-stage *Plasmodium vivax* malaria.

1586

EVALUATION OF FUNCTIONAL IMMUNOGENICITY OF *PLASMODIUM FALCIPARUM* TRANSMISSION-BLOCKING ANTIGEN PFS25 PRODUCED IN *E. COLI* ADJUVANTED IN VARIOUS NANOPARTICLES AND ADJUVANTS

Rajesh Kumar[1], Geetha Bansal[1], Grace Ledet[2], Richard Graves[2], Tarun Mandal[2], Dibyadyuti Datta[1], Evelina Angov[1], Nirbhay Kumar[1]

[1]Tulane University School of Public Health & Tropical Medicine, New Orleans, LA, United States, [2]Center for Nanomedicine and Drug Delivery, Xavier University College of Pharmacy, New Orleans, LA, United States

Pfs25, expressed on the surface of gametes, zygotes and ookinetes, is an established target antigen for malaria transmission blocking vaccines. Previously, we have reported that codon harmonized recombinant Pfs25 (CH-rPfs25) produced in E. coli elicited highly potent transmission blocking antibodies using Montanide ISA51, Alum and CFA as adjuvants. In the current study, we have undertaken extensive evaluation of various nanoparticles/adjuvants via different routes of immunization to identify safer and effective adjuvants. Mice were immunized with CH-rPfs25 via IM route (10 µg, three doses at 4 week intervals). Mice immunized with CHrPfs25 in Alum via IP and IM routes induced comparable antibody titers (640,000). Since the protein was equally immunogenic by IP and IM routes, other adjuvant formulations were tested by IM route only. CH-rPfs25 was adsorbed to nano-emulsions (4% & 8%NE) and PLGA particles (2 different amounts). CH-rPfs25 formulated in 4% NE gave highest antibody response (ELISA titer 1,280,000) as compared to 320,000 in 8% NE. Antibody titers with PLGA (10 and 20 mg PLGA) were only 160,000. We also evaluated NE formulations combined with MPLA and chitosan, and both demonstrated 640,000 antibody titers. Functional activity of antibodies was evaluated by standard membrane feeding assay using purified IgG (50- 400 µg/ml) from immunized animals. 100 % transmission blocking activity (no oocysts detected) was observed at 400 ug/ml of IgG from Alum group (both routes IP and IM), NE (4%), and NE-MPLA. Purified IgG from various adjuvant groups at lower doses (100 µg/ml) still exhibited >90% transmission blocking activity, while 52-81% blocking was seen at 50 ug/ml. Our results suggest that CH-rPfs25 is strongly immunogenic by different routes and as formulations with Alum and NE. We are continuing these studies to develop effective vaccine formulations for further evaluation and investigations into immune correlates of relative immunogenicity of CH-rPfs25 in various adjuvants.

1587

ENHANCING PRE-ERYTHROCYTIC STAGE VACCINE EFFICACY WITH THE DEVELOPMENT OF A HIGHLY IMMUNOGENIC VIRUS-LIKE PARTICLE VACCINE AND A MULTI-COMPONENT VACCINE STRATEGY

Katharine A. Collins, Matthew Cottingham, Rebecca Hillson, Sarah Gilbert, Adrian V. Hill

University of Oxford, Oxford, United Kingdom

CSP-based subunit vaccines have been shown to protect against malaria in a range of models, but to date, none have been able to elicit high levels of durable sterilising efficacy in human field trials. Our aim is to enhance pre-erythrocytic stage vaccine efficacy by two methods. Firstly by increasing the immunogenicity of a CSP-based virus-like particle (VLP) vaccine, and secondly by combining the VLP vaccine with a liver-stage viral vector vaccine regimen. To enhance the immunogenicity of the CSP-based particle we have developed an improved RTS,S like vaccine called R21. RTS,S is the leading CSP-based vaccine and consists of particles formed from a mixture of two proteins, with only ~20% of the total protein content being a CSP-HBsAg fusion protein. In the R21 particles, 100% of the total protein content is a CSP-HBsAg fusion protein and hence R21 will contain a much higher percentage of CSP than RTS,S. This could result in enhanced immunogenicity and efficacy and is currently under evaluation. The immunogenicity and efficacy of R21 + adjuvant was compared to non-particulate recombinant CSP + adjuvant in BALB/c mice. R21 was found to be more immunogenic and induced 10 fold greater levels of anti-CS antibodies as well as higher frequencies of CS specific T cells than CSP. These serum antibody titres were also durable and were maintained at high titres when measured 3 months after vaccination. R21 was also significantly more protective than non-particulate CSP in a BALB/c model against *P. berghei* transgenic for *P. falciparum* CSP. Vaccination with R21 + Matrix M sterilely protected 82.5% of mice compared to only 42.5% with CSP + Matrix M ($p = 0.014$). R21 was also assessed in combination with the ChAd63 ME.TRAP - MVA ME.TRAP vaccine regimen and there was no interference with the induction of vaccine specific immune responses when the vaccines were mixed and administered together. In addition, sterile efficacy against sporozoite challenge was significantly enhanced in the mixed vaccine group. R21 is now being taken forward for evaluation in Phase I/IIa clinical trials.

1588

PROJECTED COST-EFFECTIVENESS OF RTS,S VACCINATION IN 43 SUB-SAHARAN AFRICAN COUNTRIES

Katya Galactionova, Fabrizio Tediosi, Thomas A. Smith, Melissa A. Penny

Swiss Tropical and Public Health Institute, Basel, Switzerland

Phase III trials of the RTS,S malaria vaccine are generating new estimates of its efficacy and decay rate. To assess the appropriateness of introducing RTS,S into the EPI in endemic African countries cost-effectiveness estimates need to be updated with the most recent clinical efficacy data. We used methods for rapidly updating projections of country specific public health impact of the vaccine, and combined these with costing models for malaria case management and immunization to assess the cost-effectiveness of RTS,S introduction. We consider deployment with a 3 dose schedule targeting infants 6, 10 and 14 weeks of age, an older cohort vaccinated at 6, 7.5 and 9 months, and a 4 dose schedule as above, which includes a booster at 18 months after the third dose. Allowing for differences in epidemiological context and health systems we generate

predictions tailored to countries examined and apt to inform malaria control policy. An ensemble of individual-based stochastic simulation models of *Plasmodium falciparum* dynamics, with varied assumptions about immune decay, transmission heterogeneity, and access to treatment fit to an extensive library of field data were used to predict the impact of RTS,S. For each country average and incremental cost-effectiveness ratios were calculated relative to the routine case management and alternate vaccine deployment strategies. We show that RTS,S is likely to be a highly cost-effective intervention with a significant impact on malaria burden in endemic settings and cost per DALY averted generally comparable to routine malaria control interventions. Depending on vaccine properties, coverage, and age-related disease burden vaccinating younger cohorts may avert more disease and at a lower cost compared to older age groups, even if initial efficacy is lower. As the vaccine targets only a small fraction of the population susceptible to malaria and provides limited protection it does not eliminate the need for other control programs. Access to effective treatment is particularly important to sustain health gains achieved with the RTS,S.

1589

METHODOLOGY TO ESTIMATE THE COST OF INTRODUCING RTS,S VACCINE INTO A NATIONAL IMMUNIZATION PROGRAM IN SUB-SAHARAN AFRICAN COUNTRIES

Katya Galactionova, Fabrizio Tediosi

Swiss Tropical and Public Health Institute, Basel, Switzerland

Having demonstrated moderate levels of efficacy in Phase II and III trials in Africa, the RTS,S vaccine against *P. falciparum* malaria is currently considered for use within the Expanded Programme on Immunization in a large number of endemic settings. Introduction of a new vaccines demands extensive resources from the health system. Aside from the vaccine and related immunization supplies, resources are required for storage and supply chain, training, development of educational materials, social mobilization, supervision, monitoring, vaccine delivery, and waste management. We propose a generalizable methodology to estimate these costs related to vaccine introduction in African countries. Costs are evaluated from a broad provider perspective using the ingredients approach; these reflect the economic value of resources, and take into account overheads and cost of inputs shared with other health interventions. To address the uncertainty about the level of existing capacity in the health system we consider several states of the EPI: no spare capacity, estimated current capacity, and sufficient spare capacity to accommodate the new vaccine. At each capacity level we develop a series of normative scenarios for service delivery and capacity scale-up in accordance with the current operational guidelines. Scenarios are adapted to a given country setting to take into account among other the structure of the EPI program, distribution model, geography, and population dynamics. Resource lists and quantity assumptions defined for each immunization scenario are matched with price and unit cost data via cost functions to assess the overall cost of the program. The methodology takes advantage of country data on prices of key inputs using routinely collected data from the cMYP, UNICEF, and WHO-CHOICE. The methodology is applied to assess cost of RTS,S introduction in 6 endemic countries. We test the robustness of estimates generated by varying core assumptions and prices of key inputs and validate against the literature on cost of EPI program and introduction of other new vaccines.

1590

EFFICACY, SAFETY AND IMMUNOGENICITY OF HETEROLOGOUS PRIME-BOOST IMMUNIZATION WITH THE CANDIDATE MALARIA VACCINES CHAD63 ME-TRAP AND MVA ME-TRAP IN 5-17 MONTHS OLD BURKINABE INFANTS AND CHILDREN

Alfred B. Tiono[1], Issa N. Ouedraogo[1], Jean-Baptist Yaro[1], Katie Ewer[2], Edith C. Bougouma[1], Issiaka Soulama[1], Rachel Roberts[2], Amidou Diarra[1], Alphonse Ouedraogo[1], Alison Lawrie[2], Sam Coulibaly[1], Thomas Rampling[2], Carly Bliss[2], Aïssata Barry[1], Ines Petersen[3], Georgina Bowyer[2], Nick Anagnostou[2], Nicola Viebig[3], Egeruan Babatunde Imoukhuede[2], Philip Bejon[4], Adrian Hill[2], Sodiomon B. Sirima[1]

[1]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso, [2]University of Oxford, Oxford, United Kingdom, [3]European Vaccine Initiative, Heidelberg, Germany, [4]Kenya Medical Research Institute, Kilifi, Kenya

The development of an effective vaccine against malaria is a high priority and of great importance in the context of coordinated efforts to reduce the burden of malaria. The protective efficacy of the candidate malaria vaccines ChAd63 ME-TRAP and MVA ME-TRAP is being evaluated in an ongoing phase I/IIb double-blind randomized trial. This immunization regime has shown significant partial efficacy in controlled human malaria infection trials and an initial time-to-infection trial in adults in Kenya. Initial efficacy results from this first efficacy trial in African infants will be available in May 2014. The primary objective is to evaluate the protective efficacy against clinical malaria, for a period of 6 months after the last vaccination. Clinical malaria is defined as fever (axillary temperature ≥37.5°C) together with *Plasmodium falciparum* count > 5,000 p/µl. Seven hundred infants and children aged 5-17 months were randomized in 1:1 ratio to receive either ChAd63 ME-TRAP / MVA ME-TRAP in prime-boost immunization or rabies vaccine. Immunization schedule was 0, 8 weeks. Clinical malaria episodes were captured through a health-facility based passive case surveillance method. Venous blood samples for cellular and humoral immunogenicity were collected at various timepoints. Vaccine efficacy will be assessed using Cox regression models. For analysis of first or only episodes of *P. falciparum* malaria, the incidence of episodes for each group will be presented. Secondary analysis will examine multiple episodes, using the robust clustering method by individual. Analysis of vaccination immunogenicity will describe the arithmetic and geometric means and median spots per million PBMC by vaccine group and timepoints. The safety analysis will include all solicited and unsolicited local and systemic adverse events including clinically significant laboratory abnormalities, and serious adverse events. These results will help define the potential role of these recombinant viral vectors, either used alone or as part of a multi-component vaccine, in malaria control in Africa.

1591

ANTIBODIES AGAINST A *PLASMODIUM FALCIPARUM* RHOPTRY NECK PROTEIN PFRON12 INHIBIT MEROZOITE INVASION INTO ERYTHROCYTES

Daisuke Ito[1], Eizo Takashima[1], Shinya Hatano[1], Tsutomu Yamasaki[2], Tomoyuki Hasegawa[1], Kazutoyo Miura[3], Amporn Thongkukiatkul[4], Carole A. Long[3], Jetsumon Sattabongkot[5], Motomi Torii[6], **Takafumi Tsuboi**[1]

[1]Ehime University, Matsuyama, Ehime, Japan, [2]Okayama University of Science, Okayama, Japan, [3]National Institute of Allergy and Infectious Diseases, Rockville, MD, United States, [4]Burapha University, Chonburi, Thailand, [5]Mahidol University, Bangkok, Thailand, [6]Ehime University, Toon, Ehime, Japan

Proteins coating *Plasmodium* merozoite surface and secreted from its apical organelles are considered as promising vaccine candidates for the blood-stage malaria. The rhoptry neck protein 12 of *Plasmodium falciparum* (PfRON12) was recently reported as a protein specifically

expressed in schizont and localized to the rhoptry neck of merozoite by immunoelectron microscopy (IEM). The characteristics of the PfRON12 suggest that it is a potential blood-stage vaccine candidate, and here we assessed its potential in this regard. We expressed a recombinant PfRON12 protein by the wheat germ cell-free system to obtain anti-PfRON12 antibody. Immunoblot analysis of schizont lysate detected a single band at approximately 40 kDa under reducing condition, consistent with the predicted molecular weight. In contrast, the anti-PfRON12 antibody recognized a single band at approximately 80 kDa under non-reducing condition, consistent with two-fold molecular weight of its reduced form, suggesting the native PfRON12 forms a disulfide-bond-mediated homodimer. Immunofluorescence assay and IEM revealed that PfRON12 localized to the rhoptry neck of merozoite in schizonts and to the surface of free merozoites. The biological activity of anti-PfRON12 antibody was tested by an *in vitro* growth inhibition assay, and the antibody significantly inhibits the merozoite invasion of erythrocytes. Since anti-PfRON12 antibody inhibited the merozoite invasion *in vitro*, we decided to investigate whether PfRON12 is exposed to the human immune system in *P. falciparum*-infected individuals. The sera from *P. falciparum* infected individuals in Thailand and Mali reacted with the recombinant PfRON12, indicating PfRON12 is immunogenic in humans. Our findings suggest that PfRON12 plays an important role in the merozoite invasion process, and that it merits an additional evaluation as a *P. falciparum* blood-stage vaccine candidate.

1592

ADMINISTRATION OF PFSPZ VACCINE BY DIRECT VENOUS INOCULATION IN AFRICA

Freda Omaswa[1], Sara Healy[2], Mahamadou S. Sissoko[3], Abdoulaye Katile[3], Amagana Dolo[3], Karamoko Niare[3], Amadou Niangaly[3], Peter Billingsley[4], Anita Manoj[4], Eric James[4], B. Kim Lee Sim[4], Michael Walther[2], Ogobara Doumb Doumbo[3], Patrick Duffy[2], Stephen L. Hoffman[4]

[1]University of British Columbia, Vancouver, BC, Canada, [2]Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [3]Malaria Research and Training Center, Mali-NIAID ICER, University of Science, Techniques and Technologies of Bamako, Bamako, Mali, [4]Sanaria Inc., Rockville, MD, United States

Malaria is a major cause of morbidity and mortality despite considerable investment in existing anti-malarial measures, highlighting the need for a vaccine. The vaccine candidate, Sanaria® PfSPZ Vaccine, contains radiation-attenuated, aseptic, purified, cryopreserved *Plasmodium falciparum* (Pf) sporozoites (SPZ). Healthy 18-35 year Malians were enrolled in a randomized, double-blind, placebo-controlled trial to assess safety, tolerability, immunogenicity, ease of administration, and protective efficacy against naturally occurring malaria of PfSPZ Vaccine administered by direct venous inoculation (DVI). PfSPZ Vaccine was thawed, diluted to a 0.5 mL volume in a 1 mL syringe with a 25-gauge needle, and then passed to local physicians for administration. Local and systemic reactogenicity were solicited through 7 days after each vaccination. A survey on subject perception of vaccination procedure will be administered at the end of the vaccination phase in July 2014. In total, 105 volunteers have been vaccinated, 12 were enrolled in a pilot safety group and 93 were randomized to receive 5 doses of $2.7x10^5$ PfSPZ or normal saline placebo. At this time, 207 DVIs with PfSPZ Vaccine or placebo have been administered. The time from vaccine request by the clinical team to thaw and formulation in a syringe to completion of injection was on average 6 minutes and injections on average took < 10 sec. Of the 207 vaccinations, only one has required a second injection attempt. This was for inability to locate a vein for the 1st dose in the pilot group. Vaccinations have been well tolerated with no local reactogenicity. Seven episodes of solicited systemic reactogenicity, all grade 1, have been reported. Complete data on safety, tolerability, and administration after 5 doses of PfSPZ Vaccine will be presented. DVI administration of PfSPZ has been rapid, efficient and extremely well-tolerated. This is a 1st step toward establishing the conditions for operational implementation and logistics for mass administration of PfSPZ Vaccine in Africa.

1593

CHLOROQUINE NEITHER ELIMINATES NOR DELAYS LIVER STAGE DEVELOPMENT OF *PLASMODIUM* DURING CHEMOPROPHYLAXIS VACCINATION

Tejram Sahu[1], Lynn E. Lmbert[2], Jessica Hinderer[2], Solomon Conteh[1], Sachy Orr Gonzalez[2], Patrick E. Duffy[1]

[1]National Institute of Allergy and Infectious Diseases, Rockville, MD, United States, [2]National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States

Chloroquine (CQ) has been used in Chemoprophylaxis Vaccination (CVac) against malaria, whereby sporozoite inoculations under the umbrella of CQ prophylaxis induce liver stage-specific immunity and long-lasting sterile protection against homologous parasite strain. CQ is known to kill blood stage parasites, but its effect on liver stages is poorly studied, and the mechanism by which CVac-CQ induces strong protective immunity is not understood. We used a luciferase expressing rodent parasite, *Plasmodium yoelii*-Luc (Py-Luc), to monitor the effect of CQ on *Plasmodium* liver stage development. Balb/c mice with or without CQ prophylaxis were infected with Py-Luc sporozoites. Primaquine (PQ), a liver stage-specific anti-parasitic drug, was included as a positive control. We followed parasite development by intra-vital imaging at 44h, 54h and 60h post-infection. Parasite burden in liver was measured by quantifying bioluminescence of whole body and isolated livers, as well as by quantifying liver stage parasite transcripts by qRT-PCR. Delay in appearance of parasites in the blood was monitored by microscopic observation of Giemsa-stained thin blood smears. The parasite load in livers of CQ treated and untreated mice did not differ ($p=0.714$), and this was consistent at all three timepoints. PQ treated mice had a significant reduction in parasite burden as compared to both CQ treated and untreated groups ($p=0.008$), and were similar to the non-infected control mice. Parasites appeared in the blood stream of both CQ treated and untreated mice at the 54h time point. Taken together, our findings indicate that CQ neither eliminates liver stage parasites nor delays their development. Further investigations into the mechanisms by which CVac-CQ induces protective immunity are required, and may give insights relevant to drug and vaccine development.

1594

DEVELOPMENT OF A METABOLICALLY ACTIVE, NON-REPLICATING, ASEPTIC, PURIFIED, CRYOPRESERVED, GENETICALLY ATTENUATED *PLASMODIUM FALCIPARUM* SPOROZOITE VACCINE-PFSPZ (Δ*SLARP*Δ*B9*) VACCINE

Adam M. Richman[1], B. Kim Lee Sim[1], Ben C. van Schaijk[2], Takeshi Annoura[3], Ivo H. Ploemen[2], Tao Li[1], Abraham Eappen[1], Patricia de la Vega[1], Sumana Chakravarty[1], Adam J. Ruben[1], Shahid M. Khan[3], Chris J. Janse[3], Robert W. Sauerwein[2], Stephen L. Hoffman[1]

[1]Sanaria Inc., Rockville, MD, United States, [2]Department of Medical Microbiology, Nijmegen, Netherlands, [3]The Leiden Malaria Research Group, Parasitology, Leiden, Netherlands

Sanaria® PfSPZ Vaccine, composed of radiation attenuated, aseptic, purified, cryopreserved *Plasmodium falciparum* (Pf) sporozoites (SPZ) protected 6/6 (100%) of volunteers, who received the highest dose. By mid 2014 PfSPZ Vaccine will be in clinical trials in the U.S. and 5 countries in Africa and Europe. It is intended for elimination campaigns and prevention of malaria in travelers. There would be potential manufacturing, potency and regulatory advantages if radiation-attenuated parasites were replaced with genetically attenuated parasites (GAPs). We recently reported on a new target gene, *b9* and its deletion in combination with the *slarp* gene. In rodent malarias Δ*slarp*Δ*b9* SPZs elicit excellent protective immunity and do not lead to blood stage infection.

Elimination of Pf *slarp* and *b9* genes leads to attenuation similar to radiation. All prior Pf GAPs showed leaky attenuation and breakthrough liver stage development *in vivo* or *in vitro*. After characterization in Nijmegen and Leiden, the parasites were transferred to Sanaria where master and working cell banks (MCB and WCB) were made and an engineering production run performed to demonstrate that Pf*Δslarp*Δ*b9* GAP was suitable for producing aseptic, purified, cryopreserved PfSPZ. The parasites demonstrated all growth characteristics necessary for cGMP production. The Pf*Δslarp*Δ*b9* SPZ were assayed in Sanaria's 6-day hepatocyte attenuation, 3-day hepatocyte potency, and sporozoite membrane integrity (viability) assays. For the 6-day assay we used Pf wild type and Pf*Δp52*Δ*p36* SPZ as positive controls; the wild type Pf, Pf*Δp52*Δ*p36*, and Pf*Δslarp*Δ*b9* SPZ produced 21±1, 1.5±1.25 and 0, 6-day liver stage schizonts respectively. Pf*Δslarp*Δ*b9* SPZ were potent and viable. We will next use this genetically attenuated double-mutant parasite (Pf*Δslarp*Δ*b9)* to manufacture, characterize and release a corresponding PfSPZ Vaccine, PfSPZ (*Δslarp*Δ*b9*) Vaccine (also known as PfSPZ-GA1 Vaccine) in compliance with cGMPs, conduct pre-clinical studies, submit to the appropriate U.S. and Dutch regulatory agencies, and conduct a clinical trial.

1595

FINE MAPPING OF ANTIBODY ISOTYPES AND IMMUNODOMINANT B CELL EPITOPES INDUCED BY MALARIA VACCINE, PFCELTOS/GLA-SE

Casey K. Storme[1], Sidhartha Chaudhury[2], Sathit Pichyangkul[3], Susan L. Baldwin[4], Elizabeth Duncan[1], Heather Hosie[1], Darrick Carter[4], Steven G. Reed[4], Anders Wallqvist[2], Christian F. Ockenhouse[1], Jessica Cowden[1], Elke Bergmann-Leitner[1], Randall F. Howard[4], Evelina Angov[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Biotechnology HPC Software Applications Institute, Telemedicine and Advanced Technology Research Center, Fort Detrick, MD, United States, [3]Armed Forces Research Institute of Medical Sciences, U.S. Army Medical Component, Bangkok, Thailand, [4]Infectious Disease Research Institute, Seattle, WA, United States

The secreted malarial protein Cell-Traversal protein for Ookinetes and Sporozoites (CelTOS) is highly conserved among *Plasmodium* species, essential to host hepatocyte invasion, and critical to malaria pathogenesis. We previously reported that immunization of mice with full-length recombinant CelTOS from *P. falciparum* (*Pf*CelTOS) adjuvanted in Montanide ISA-720 achieved 60% heterologous protection against a *P. berghei* sporozoite challenge. The immune mechanisms leading to this cross-species protection are based on both cellular and humoral effector mechanisms. Formulating *Pf*CelTOS with the clinically relevant adjuvant GLA-SE resulted in similar levels of protection in mice against challenge as seen with ISA-720, and these studies provided evidence to support its evaluation in a Phase 1 safety, immunogenicity with Controlled Human Malaria Infection (CHMI) clinical trial. Since protection is in part mediated by antibodies, we sought to identify protective B-cell epitopes and, thereby, we determined the antibody fine specificity of preclinical and clinical samples for *Pf*CelTOS. To this end, various protein fragments were generated in *E. coli* or as synthesized peptides, and their reactivity with sera from *Pf*CelTOS/GLA-SE immune mice, rats, non-human primates and human subjects was determined. We are currently implementing *in vitro* functional assays such as inhibition of sporozoite gliding motility and the inhibition of sporozoite invasion and development within hepatocytes (ILSDA) to identify whether this vaccine formulation induces responses to functional B-cell epitopes within the identified immunodominant regions of CelTOS. Such characterizations will determine the role of these epitopes in mediating antibody-mediated protection from preclinical and clinical studies of the CelTOS antigen.

1596

GENETIC DIVERSITY OF THE PLACENTAL MALARIA VACCINE CANDIDATE VAR2CSA IN TWO MALARIA ENDEMIC SETTINGS IN AFRICA USING PACBIO SEQUENCING

Antoine Dara[1], Mark A. Travassos[1], Matthew Adams[1], Sarah Schaffer DeRoo[1], Joana C. Silva[2], Christopher V. Plowe[1], Miriam K. Laufer[1]

[1]Howard Hughes Medical Institute/Center for Vaccine Development, University of Maryland, Baltimore, MD, United States, [2]Institute of Genome Sciences and Department of Microbiology and immunology, University of Maryland, Baltimore, MD, United States

Pregnancy-associated malaria, a leading cause of maternal anemia and low birth weight, is characterized by the sequestration of *Plasmodium falciparum*-infected erythrocytes in the placental microvasculature due to expression of VAR2CSA. Epidemiological and serological studies indicate that VAR2CSA is a target of naturally acquired immunity, suggesting that VAR2CSA could be a potential target for a pregnancy-associated malaria vaccine. However, based on limited data, there appears to be extensive genetic diversity within the var2csa gene that must be taken into account in the design of an effective vaccine. Genetic diversity has prevented the successful sequencing of var2csa from field isolates using standard sequencing platforms. Due to the low sequence complexity and high variant diversity of var2csa, applying a traditional Sanger sequencing strategy on field samples is inefficient and costly. To overcome this obstacle, we are characterizing var2csa genetic diversity in malaria parasite isolates from two different endemic regions in Africa using a combination of long range PCR amplification and the Pacific Bioscience next generation sequencing platform. We performed a multiple alignment of the publicly available 20 coding sequences and 12 upstream promoter region sequences of var2csa, and designed primers based on three of the 40 identified conserved regions with a minimum length of 25 nucleotides. A first set of primers targets a 5 kilobase (kb) region spanning the upstream promoter region to the DBLepam4 domain, and a second primer set targets a 5 kb region spanning the DBLpam3 domain to the intracellular acidic terminal segment region. We have successfully amplified the two fragments for the reference strains 3D7 and NF54 and the first fragment from the Dd2 and HB3 strains. We are employing this approach to determine the var2csa sequence of clinical isolates and characterize the extent of natural diversity in var2csa in Mali and Malawi.

1597

SIMULATION OF B CELL AFFINITY MATURATION EXPLAINS ENHANCED ANTIBODY CROSS-REACTIVITY INDUCED BY THE POLYVALENT MALARIAL VACCINE AMA1

Sidhartha Chaudhury, Jaques Reifman, Anders Wallqvist

BHSAI, U.S. Army Medical Research and Materiel Command, Fort Detrick, MD, United States

Polyvalent vaccines use a mixture of antigens representing distinct pathogen strains to induce an immune response that is cross-reactive and protective. However, such approaches often have mixed results, and it is unclear how polyvalency alters the fine specificity of the antibody response and what those consequences might be for protection. Here, we present a coarse-grain theoretical model of B cell affinity maturation during monovalent and polyvalent vaccinations that predicts the fine specificity and cross-reactivity of the antibody response. We stochastically simulate affinity maturation using a population dynamics approach where the host B cell repertoire is represented explicitly, and individual B cell sub-populations undergo rounds of stimulation, mutation, and differentiation. Antigens contain multiple epitopes and are present in subpopulations of distinct pathogen strains, each with varying degrees of cross-reactivity at the epitope level. This epitope and strain-specific model of affinity maturation enables us to study the composition of the polyclonal response in granular detail and identify the mechanisms driving serum specificity and cross-reactivity. We applied this approach to predict the antibody

response to a polyvalent vaccine based on the highly polymorphic malarial antigen AMA1. Our simulations show that polyvalent AMA1 vaccination induces an enhanced cross-reactive antibody response primarily through a shift in affinity maturation that favors B cells specific to shared and cross-reactive epitopes, and demonstrates how a polyvalent vaccine with a small number of strains and only moderate allelic coverage may be broadly neutralizing. These results present broad implications for general polyvalent vaccine design.

1598

HEPATITIS B, HEPATITIS C AND HIV INFECTION FREQUENCIES AMONG VOLUNTEERS SCREENED FOR MALARIA VACCINE CLINICAL TRIALS IN MALI

Merepen A. Guindo[1], Moussa L. Diakite[1], Boucary Ouologuem[1], Souleymane Traore[1], Amadou Konate[1], Abdoulaye Katile[1], Yacouba Samake[1], Bourama Kamate[1], Sarro Yeya[2], Mahamadoun H. Assadou[1], Mahamadou S. Sissoko[1], Issaka Sagara[1], Boubacar Traore[1], Joseph P. Shott[3], Sara A. Healy[4], Stephen L. Hoffman[5], Patrick E. Duffy[4], Ogobara Doumbo[1]

[1]Malaria Research and Training Center, Mali-NIAID ICER, University of Science, Techniques and Technology, Bamako, Mali, [2]SEREFO, University of Science, Techniques and Technology, Bamako, Mali, [3]Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [4]Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, Rockville, MD, United States, [5]Sanaria Inc., Rockville, MD, United States

Two clinical trials of malaria vaccine candidates (transmission blocking vaccine Pfs25-EPA/Alhydrogel® in collaboration with LMIV-NIAID) and whole organism vaccine PfSPZ (in collaboration with LMIV-NIAID and Sanaria) have begun in Bancoumana and Doneguebougou, Mali. Both villages are located within 70km of Bamako, but are different in terms of ethnicity, surrounding terrain, and primary employment. We have screened 509 men and women volunteers aged 18-45 years in Bancoumana and 18-35 years in Doneguebougou. Screening has been conducted to exclude those with human immunodeficiency virus (HIV) 1 and 2, hepatitis B virus (HBV; by hepatitis B surface antigen (HBsAg)) and hepatitis C (HCV; by anti-HCV core antibodies)). For HCV and HBV testing, AccuDiag ELISA was used in Doneguebougou and standard rapid diagnostic tests (RDT) in Bancoumana. Determine HIV1/2 Alere® RDT with positivity confirmation by ELISA GENSCREEN ULTRA Ag-Ab were used at both sites for HIV 1/2. Ten of 204 (4.9%; 95%CI [2.37-8.82]), and ten of 218 (4.6%; 95% CI [2.22-8.27]) volunteers were positive by HIV RDT respectively in Doneguebougou and Bancoumana. Four (2.0%; 95% CI [0.54-4.94])and five (2.3%; 95% CI [0.75-5.27]) were confirmed positive by HIV ELISA GENSCREEN ULTRA Ag-Ab. HIV prevalence rates were comparable *(p> 0.05)* at the two sites. Forty-six volunteers (22.5%; 95% CI [17.01-28.90]) and 21 volunteers (9.6%; 95% CI [6.06-14.35]) were HBsAg positive respectively in Doneguebougou and Bancoumana, while six (2.9%; 95% CI [1.08-6.29]) and four (1.8%; 95%CI [0.5-4.63]) were anti-HCV positive at the sites respectively. The HBsAg positive frequency in Doneguebougou is higher than in Bancoumana, and higher than in previous studies, indicating that the prevalence of HBV infections may be increasing in this area. Together these viral diseases are an important consideration in the screening process for malaria vaccine trials.

1599

PHASE 1 MALARIA VACCINE STUDIES IN AFRICA: WHAT DEFINES A NORMAL, HEALTHY VOLUNTEER?

Sara A. Healy[1], Issaka Sagara[2], Mahamadou S. Sissoko[2], Merepen A. Guindo[2], Freda Omaswa[3], Joseph P. Shott[4], Stephen L. Hoffman[3], Ogobara Doumbo[2], Patrick E. Duffy[1]

[1]Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [2]Malaria Research and Training Center, Mali-NIAID ICER, University of Science, Techniques and Technologies of Bamako, Bamako, Mali, [3]Sanaria, Inc., Rockville, MD, United States, [4]Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

As the malaria vaccine landscape continues to expand with new candidates entering phase 1 trials in Africa, the concept of a normal, healthy volunteer becomes difficult to consistently and accurately define in these settings. Inclusion/exclusion criteria and toxicity grading for these studies can vary significantly between sites, impacting adverse event reporting, study stopping criteria, immunogenicity responses, known susceptibility to malaria infection, and severity of disease. From May 2013 to February 2014, 509 volunteers were screened for either a phase 1 malaria transmission blocking vaccine trial or a phase 1 whole sporozoite malaria vaccine trial in Mali. All volunteers were screened by history, physical examination, and standard laboratory testing (hematological and biochemical parameters, urinalysis, Hepatitis B/C virus and HIV testing) with consistent screening ratios of 2 to 2.5 volunteers screened to 1 volunteer enrolled. However, other exclusion criteria, such as sickle cell disease/trait, electrocardiogram abnormalities, helminthiases, schistosomiasis, and syphilis were not consistently evaluated nor universally managed prior to enrollment, potentially creating variability in adverse event reporting. The impact on immunogenicity responses, malaria infection, and disease severity given the inconsistency in the definition of a healthy volunteer in this population prior to enrollment, is to be determined in these trials. Defining standard inclusion and exclusion criteria increases the likelihood of producing reliable and reproducible results, but must be closely balanced with the research being representative of the general healthy population under study. The creation of extremely narrow inclusion exclusion criteria can have significant implications on the ability of the study to be representative of the population under study and generalizability of the research results.

1600

A GENOMIC EPIDEMIOLOGY APPROACH TO ASSESSING AND IMPROVING STRAIN-SPECIFIC WHOLE ORGANISM VACCINE EFFICACY

Kara Moser[1], Mark Travassos[2], Amed Ouattara[2], Elliot Drábek[1], Cesar Arze[1], Claire M. Fraser[1], Kim Lee Sim[3], Stephen L. Hoffman[3], Ogobara K. Doumbo[4], Mahamadou A. Thera[4], Shannon Takala-Harrison[2], Joana C. Silva[1], Christopher V. Plowe[2]

[1]Institute for Genome Sciences, University of Maryland, Baltimore, MD, United States, [2]Howard Hughes Medical Institute/Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States, [3]Sanaria Inc., Rockville, MD, United States, [4]Malaria Research and Training Center, University of Sciences, Techniques and Technology of Bamako, Bamako, Mali

Genetic diversity in *Plasmodium falciparum* is an obstacle for broadly efficacious malaria vaccines. A subunit blood stage malaria vaccine provides allele-specific efficacy, preventing only clinical malaria caused by parasites identical to the vaccine antigen at key polymorphic loci. A similar phenomenon may apply to whole-organism vaccines, albeit potentially at many, currently unknown, loci throughout the parasite genome. The attenuated whole-organism malaria vaccine PfSPZ Vaccine, which protects against homologous challenge, is based on the African strain NF54, the parent stock of the reference *P. falciparum* strain, 3D7. Controlled human

malaria infection trials to assess heterologous protection will initially use the South American strain 7G8. To determine how NF54 and 7G8 relate to genetic variation in natural populations, whole genome sequence data from NF54, 7G8 and from twelve mono- or polyclonal clinical samples of *P. falciparum* from a Malian village were analyzed. SNPs for all 12 Malian strains, NF54 and 7G8 were called against the 3D7 genome using GATK. Using the most reliable of the SNP filters tested, an average of 37,000 SNPs were called for Malian strains. In contrast, 383 and 17,667 SNPs were called for NF54 and 7G8, respectively. As expected, NF54 was nearly identical to 3D7, and the Malian strains were on average considerably more dissimilar genetically from 3D7, and by proxy from NF54, than is 7G8. Principal coordinate analysis was used to compare genetic diversity between strains, both at the genome-wide level and in a subset of 26 single-copy antigenic genes. This analysis placed NF54 centrally among the 12 Malian samples, suggesting a fairly representative genetic composition. NF54 and 7G8 clustered tightly with one another and with a subset of the monoclonal strains, possibly representing an artifact of polyclonality. Principal coordinate analysis of single-copy antigens showed some of the field isolates to be more distant from NF54 than 7G8 is, suggesting that additional suitable challenge strains can be easily identified.

1601

HUMANIZED DRAG MICE SUSTAIN THE VERTEBRATE LIFE CYCLE OF *PLASMODIUM FALCIPARUM* AND ELICIT PARASITE-SPECIFIC IMMUNE RESPONSES

Wathsala Wijayalath[1], Sai Majji[1], Eileen F. Villasante[1], Teodor D. Brumeanu[2], Thomas L. Richie[1], Sofia Casares[1]

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

Plasmodium falciparum is one of the deadliest protozoan parasites among the five species of human malaria, which accounts for the highest morbidity and mortality in tropical and sub-tropical countries. In addition of having multiple life cycle stages in the human host, *P. falciparum* parasites shows high antigenic diversity and cytoadherence properties, which increases the severity and complexity of the disease. Numerous efforts have been conducted over decades to address disease pathogenesis, immunity, and vaccine development, mostly in in-vitro or in rodent or non-human primate models. However, none of these models completely represent the disease, as it is in the natural human host, thus demanding the necessity of developing an accurate animal model. We generated a HLA- class II expressing humanized DRAG mice, which develop a functional human immune system, following human hematopoietic stem cell infusion. DRAG mice develop human hepatocytes, kupffer cells, liver endothelial cells and erythrocytes and sustain the complete life cycle of *P. falciparum* malaria parasite. Our data also demonstrate that the infected DRAG mice self-cure blood-stage infection following intravenous inoculation of live *P. falciparum* sporozoites and elicit humoral responses characterized by IgM and IgG antibodies against ring, trophozoite and schizont stage parasites. The infected DRAG mice also elicit cellular responses mediated by TNF-alpha against *P. falciparum* blood-stage parasites. Thus the DRAG mice represent the first small animal model, which has the ability to sustain the complete *P. falciparum* life cycle and to elicit parasite-specific immune responses.

1602

INJECTION OF PURIFIED, ASEPTIC CRYOPRESERVED *PLASMODIUM FALCIPARUM* SPOROZOITES (PFSPZ CHALLENGE) IS AN ALTERNATIVE TO MOSQUITO BITE ADMINISTRATION FOR CONTROLLED HUMAN MALARIA INFECTION (CHMI)

Thomas L. Richie[1], Peter F. Billingsley[1], B. Kim Lee Sim[1], Eric R. James[1], Meta Roestenberg[2], Else M. Bijker[3], Kirsten E. Lyke[4], Matthew B. Laurens[4], Susanne H. Hodgson[5], Adrian V. Hill[5], Seif Shekalaghe[6], Salim Abdulla[6], Benjamin Mordmüller[7], Peter G. Kremsner[7], Gloria P. Gómez-Pérez[8], Almudena Legarda[8], Pedro L. Alonso[8], Bernhards Ogutu[9], Kevin Marsh[10], Robert W. Sauerwein[3], Stephen L. Hoffman[1]

[1]Sanaria, Inc., Rockville, MD, United States, [2]Leiden University Medical Center, Leiden, Netherlands, [3]Radboud University Medical Center, Nijmegen, Netherlands, [4]Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States, [5]The Jenner Institute, University of Oxford, Oxford, United Kingdom, [6]Ifakara Health Institute, Bagamoyo, United Republic of Tanzania, [7]Tropical Medicine Institute, University of Tübingen, Tübingen, Germany, [8]Barcelona Centre for International Health Research (CRESIB), Hospital Clínic - University of Barcelona, Barcelona, Spain, [9]Centre for Clinical Research, Kenya Medical Research Institute, Nairobi, Kenya, [10]KEMRI Wellcome Trust Research Programme, Kilifi, Kenya

Plasmodium falciparum (Pf) sporozoite (SPZ)-infected mosquitoes for controlled human malaria infections (CHMI) are produced routinely only in the USA and the Netherlands, by a small number of restricted-access insectaries. In the 28-day CHMI procedure, healthy adults are infected with malaria by the bites of 5 PfSPZ-infected mosquitoes and followed to assess efficacy of antimalarial drugs or vaccines. Although CHMI permits critical decisions regarding product advancement without the need for expensive and lengthy field evaluations, its use is limited by the requirement for a secure insectary capable of producing infected mosquitoes. This restricts performance of CHMI to local sites or requires transport of PfSPZ-infected mosquitoes to sites with a mosquito-secure facility. Costs may exceed $100K per CHMI. These limitations have been largely bypassed by Sanaria® PfSPZ Challenge, a cGMP product consisting of highly purified, aseptic, cryopreserved PfSPZ for parenteral use that is easily stored and transported to distant sites. Seven trials enrolling 178 volunteers have been conducted to test the safety, tolerability and infectivity of PfSPZ Challenge given by intradermal (ID), intramuscular (IM), intravenous (IV), or direct venous inoculation (DVI) routes, first in the Netherlands and subsequently in the UK, Tanzania, USA, Germany, Spain and Kenya. PfSPZ Challenge has been uniformly safe, and has infected 100% of volunteers by each route in 5 of the 7 trials using well-tolerated doses. IV, DVI and IM injection have achieved pre-patent periods of 11.0-11.5 days, matching those following mosquito bites, and IV and IM demonstrated a dose response. PfSPZ Challenge has enabled CHMI in Africa, emphasizing the potential for this "challenge in a bottle" to accelerate development of novel antimalarial drugs and vaccines and to promote understanding of innate and acquired immunity to malaria.

1603

CD8 T CELLS MEDIATE STERILE PROTECTION OF OUTBRED MICE FROM *PLASMODIUM YOELII* CHALLENGE FOLLOWING RECOMBINANT DNA-PRIME/AD5-BOOST IMMUNIZATION EXPRESSING TWO CANDIDATE VACCINE ANTIGENS

Emily C. Smith[1], Rebecca Danner[1], Jessica S. Bolton[1], Andrea K. Strein[1], Nonenipha Rangel[1], Esteban Abot[1], Arnel Belmonte[1], Noelle Patterson[1], Keith Limbach[1], Martha Sedegah[2], Eileen F. Villasante[2], Thomas L. Richie[2], Joao C. Aguiar[3]

[1]Henry M. Jackson Foundation/Naval Medical Research Center, Silver Spring, MD, United States, [2]Naval Medical Research Center, Silver Spring, MD, United States, [3]CAMRIS/Naval Medical Research Center, Silver Spring, MD, United States

Previously, we identified two antigens, UIS3 and Falstatin, which conferred sterile protection of outbred mice from *P. yoelii* sporozoite challenge following recombinant DNA-prime/Adenovirus serotype 5 (Ad5)-boost immunization. In the present study, we aimed to identify the immune component(s) mediating this protection. CD1 mice were primed with Py UIS3 and Py Falstatin DNA vectors followed by a boost with recombinant Ad5 vectors. Protection ranged from 7% to 57% in multiple experiments, with the highest level of protection observed for Ad5 boost of 10^{10} pu. Sporozoite IFA titers ranged from 1:40 to 1:640, while blood-stage titers ranged from 1:2560 to 1:20480. A wide range of ELISA titers was observed to UIS3 protein (range 1:25 to 1:78125) while those to Falstatin were more uniformly high (range 1:34576 to 1:144317). High frequencies of CD8 T cells producing IFN-g following Falstatin stimulation were observed in the spleens of immunized mice (range 9.6% to 18.9% of CD8 T cells). A fraction of the responding T cells also produced TNF and/or IL-2 in addition to IFN-g. Interestingly, the frequencies of Falstatin-specific CD8 T cells producing IFN-g were significantly increased among mice immunized with both UIS3 and Falstatin (above) compared to those immunized with Falstatin alone (range 4.7% to 7.4% of CD8 T cells, $p<0.01$). Similarly, endpoint serum ELISA titers targeting Falstatin were also increased among mice immunized against both antigens (above) compared to those immunized against Falstatin alone (range 1:15663.4 to 1:108539, $p<0.05$). *In vivo* depletion of CD8 T cells prior to challenge resulted in complete loss of the protection. These data indicate that UIS3 and Falstatin are promising candidate malaria vaccine antigens. Further study is required to fully understand the individual antigen contribution to protection and its duration, as well as to identify an optimal platform eliciting high level durable T cell immunity.

1604

D/HUAD5-PFCSLAM, A *PLASMODIUM FALCIPARUM* MULTI-ANTIGEN MULTI-STAGE ADENOVIRUS VECTORED VACCINE CANDIDATE, IS IMMUNOGENIC IN BALB/C MICE

Noelle B. Patterson[1], Maureen E. Stefaniak[1], Joseph J. Campo[1], Kalpana Gowda[1], Keith Limbach[1], Joseph T. Bruder[2], C. Richter King[2], Pamela Resch[3], Marilyn Ferrari[3], Larry R. Smith[3], Thomas L. Richie[1], Denise L. Doolan[1], Eileen Villasante[1]

[1]Naval Medical Research Center, Silver Spring, MD, United States, [2]GenVec, Inc., Gaithersburg, MD, United States, [3]Vical, Inc., San Diego, CA, United States

We have demonstrated that a malaria DNA/human adenovirus serotype 5-vectored vaccine platform (Ad) prime/boost vaccine expressing *Pf*CSP and *Pf*AMA1 antigens (*Pf*CA) is well tolerated, immunogenic, and efficacious in a Phase 1 clinical study (27%, 4/15 volunteers sterilely protected against controlled human malaria infection administered by mosquito bite) (Chuang et al., 2013). We are now evaluating strategies to increase efficacy. Here, we report the addition of antigens to broaden vaccine immunogenicity, with a focus on inducing a multi-valent, multi-stage, and multi-immune (cellular and humoral) response against both pre-erythrocytic and erythrocytic parasite life cycle stages. *Pf*CSLAM is a cocktail of *Pf*CSP, *Pf*SSP2/TRAP, *Pf*LSA1, *Pf*AMA1, and *Pf*MSP1 DNA and Ad vectors; the first four antigens are designed to induce T cell responses targeting sporozoite and liver stages, while those encoding AMA1 and MSP1 are designed to induce antibody responses targeting asexual blood stages. We have previously demonstrated that Ad and DNA/Ad prime/boost vaccines are immunogenic in murine, swine, and nonhuman primate models, and that a *Pf*CA DNA/Ad vaccine is protective in humans. Here, BALB/c mice were immunized i.m. with either the individual components, the 5 antigen *Pf*CSLAM mixture, or a 4 antigen *Pf*CLAM mixture, administered as a single Ad dose ($1x10^8$ pu) on study day (SD) 28, two Ad doses on SD1 and SD28, or pDNA (50 µg) on SD1 and Ad ($1x10^8$ pu) on SD28. Animals were bled pre-, 2 and 6 weeks post-boost for antibody assays, and spleens were harvested 2 and 6 weeks post-boost for T cell assays. Results established that the 5- and 4-antigen mixtures, CSLAM mixture ± SSP2, induced antigen-specific T cell and antibody responses to each antigen comparable to those induced by the individual components, as assessed by IFN-γ ELISpot or ELISA. These data support that the addition of antigens to the *Pf*CA mixture which is protective in humans can broaden the vaccine specificity. Future plans include GMP manufacture and clinical testing of the CSLAM or CLAM DNA prime /Ad boost vaccine.

1605

PLASMODIUM FALCIPARUM AMA1-BASED SUBUNIT VACCINE FMP2.1/AS02A ELICITS A DIVERSE AND STRONG YET UNPROTECTIVE IMMUNE RESPONSE IN A PEDIATRIC COHORT IN BANDIAGARA, MALI

Jason A. Bailey[1], Mark A. Travassos[1], Christopher G. Jacob[1], Amed Oattara[1], Drissa Coulibaly[2], Matthew B. Laurens[1], Shannon Takala-Harrison[1], Carter L. Diggs[3], Lorraine Soisson[3], Tyler S. Brown[1], Kirsten E. Lyke[1], David E. Lanar[4], Sheetj Dutta[4], D. Gray Heppner[4], Jozelyn Pablo[5], Rie Nakajima[5], Algis Jasinskas[5], Amadou Niangaly[2], Andrea A. Berry[1], Bourema Kouriba[2], Mahamadou A. Thera[2], Ogobara K. Doumbo[2], Philip L. Felgner[5], Christopher V. Plowe[1]

[1]Howard Hughes Medical Institutes, Center for Vaccine Development, Baltimore, MD, United States, [2]Malaria Research and Training Center, Bamako, Mali, [3]United States Agency for International Development, Washington, DC, United States, [4]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [5]Department of Medicine, University of California Irvine, Irvine, CA, United States

FMP2.1/$AS02_A$ is a blood stage malaria subunit vaccine candidate based on the ectodomain of apical membrane antigen 1 (AMA1) of the 3D7 strain of *Plasmodium falciparum*. In a Phase 2 vaccine trial in 400 children aged 1-6 years in Bandiagara, Mali, West Africa, the vaccine had only a statistically insignificant 17% efficacy against all clinical malaria episodes when compared to the rabies control vaccine. However, it had 64% efficacy against clinical malaria caused by homologous strains with respect to eight pre-specified polymorphic amino acid positions. To assess anti-AMA1 antibody specificity in AMA1 vaccine trials, we developed a protein microarray for measuring seroreactivity to 263 unique AMA1 ectodomain variants detected by sequencing the *ama1* gene in field samples. We evaluated AMA1 seroreactivity in a random sample of 40 children (aged 1-6 years) and 20 adults (aged 18-55 years) pre- and post-vaccination with FMP2.1/$AS02_A$ or rabies control vaccine. Both children and adults immunized with the AMA1 vaccine had broad and strong immune responses to diverse AMA1 variants compared to controls 90 days after the first immunization. Due to the broad cross-reactivity of antibodies generated by the vaccine, we were unable to pinpoint specific AMA1 variants or polymorphic epitopes associated with protection from clinical malaria. Further analysis using multivariable logistic regression, as well as principle components analysis, Random Forest, and receiver-operating characteristic curves, suggest that antibodies stimulated by the FMP2.1/$AS02_A$ vaccine may be biased, binding preferentially to immunodominant but unprotective AMA1 epitopes. This form of deceptive imprinting has been described in HIV and influenza as a tool for immune escape and also has been characterized in malaria as the 'smokescreen effect'. These

results suggest that *a priori* knowledge of a functional epitope map could inform the selection of malaria subunit vaccine antigens that would generate protective antibody populations.

1606

A FULL-LENGTH *PLASMODIUM FALCIPARUM* RECOMBINANT CIRCUMSPOROZOITE PROTEIN EXPRESSED BY PSEUDOMONAS FLUORESCENS PLATFORM AS A MALARIA VACCINE CANDIDATE

Gabriel Gutierrez[1], Amy R. Noe[1], Steve Giardina[1], Diego Espinosa[2], Ryan Haverstock[3], Jeff Allen[3], Hongfan Jin[3], Xiangming Li[4], Jordana Coelho-dos-Reis[4], Ryota Funakoshi[4], John Sacci[5], Diane Retallack[3], Tom Vedvick Vedvick[6], Chris Fox[6], Ramses Ayala[1], Steven G. Reed[6], Fidel Zavala[1], Moriya Tsuji[4]

[1]Leidos Life Sciences, Frederick, MD, United States, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [3]Pfenex Inc., San Diego, CA, United States, [4]Aaron Diamond AIDS Research Center, New York, NY, United States, [5]University of Maryland School of Medicine, Baltimore, MD, United States, [6]Infectious Disease Research Institute, Seattle, WA, United States

The circumsporozoite protein (CSP) of *Plasmodium falciparum* is a major surface protein, which forms a dense coat on the sporozoite's surface. Preclinical research on CSP and clinical evaluation of a CSP fragment-based RTS,S/AS01 vaccine have demonstrated a modest degree of protection against *P. falciparum*, mediated in part by humoral immunity and in part by cell-mediated immunity. Given the partial protective efficacy of the RTS,S/AS01 vaccine in a recent Phase 3 trial, further improvement of CSP-based vaccines is crucial. Here we describe the preclinical evaluation of a full-length, recombinant CSP (rCSP)-based vaccine candidate against *P. falciparum* malaria suitable for current Good Manufacturing Practice (cGMP) production. Utilizing a novel high-throughput Pseudomonas fluorescens expression platform, we demonstrated greater efficacy of full-length rCSP as compared to N-terminally truncated versions, rapidly down-selected a promising lead vaccine candidate, and developed a high-yield purification process to express immunologically active, intact antigen for clinical trial material production. The rCSP, when formulated with various adjuvants, induced antigen-specific antibody responses as measured by ELISA and immunofluorescence assay (IFA), as well as CD4+ T-cell responses as determined by ELISpot. The adjuvanted rCSP vaccine conferred protection in mice when challenged with transgenic *P. berghei* sporozoites containing the *P. falciparum* repeat region of CSP. Furthermore, heterologous prime/boost regimens with adjuvanted rCSP and an adenovirus type 35-vectored CSP (Ad35CS) showed modest improvements in eliciting CSP-specific T-cell responses and anti-malarial protection, depending on the order of vaccine delivery. Collectively, these data support the importance of a further clinical development of adjuvanted rCSP, either as a stand-alone product or as one of the components in a heterologous prime/boost strategy, ultimately acting as an effective vaccine candidate for the mitigation of *P. falciparum*-induced malaria.

1607

A MONOCLONAL ANTIBODY AGAINST THE N-TERMINAL REGION OF THE *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE PROTEIN STRONGLY INHIBITS SPOROZOITE INVASION OF HEPATOCYTES

Diego Espinosa[1], **Amy Noe**[2], Photini Sinnis[1], Gabriel Gutierrez[2], Fidel Zavala[1]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Leidos Life Sciences, Frederick, MD, United States

Studies in animals and human volunteers have demonstrated that antibodies against the Circumsporozoite Protein (CSP) can protect against infection by *Plasmodium* sporozoites. Due to its repetitive nature and tandem disposition, the epitopes at CSP's repeat region are the most prominent target of protective antibody responses. However, it has been long hypothesized that antibodies raised against epitopes outside the repeat domain can also confer significant protection against sporozoite invasion. Using a newly developed chimeric *Plasmodium berghei* strain bearing the N-terminal region of the *P. falciparum* CSP, we report the characterization of a monoclonal antibody (MAb) recognizing the *P. falciparum* CSP. We mapped the fine epitope specificity of this MAb (5D5) and established that it recognizes an amino acid sequence immediately adjacent to Region I of the *P. falciparum* CSP. Using both the novel *P. berghei- P. falciparum* chimera and *P. falciparum* parasites, we further characterized the 5D5 MAb epitope specificity and show that it can bind both live and air-dried fixed sporozoites. Most importantly, we demonstrate that 5D5 can strongly inhibit parasite infection *in vivo* and can inhibit cleavage of CSP, and so provide additional evidence that antibodies targeting epitopes other than those at CSP's repeat region can be highly protective. Furthermore, we propose that this MAb could be utilized as an antibody-based, therapeutic prophylaxis, which may be a critical tool in the face of growing malaria drug resistance.

1608

SAFETY AND PROTECTIVE EFFICACY OF INTRAVENOUS IMMUNIZATION WITH CRYOPRESERVED *PLASMODIUM FALCIPARUM* SPOROZOITES UNDER CHEMOPROPHYLAXIS - TUECHMI-002

Benjamin Mordmueller[1], B. Kim Lee Sim[2], Peter F. Billingsley[2], Eric R. James[2], Meral Esen[1], Markus Gmeiner[1], Guezin Surat[1], Heimo Lagler[1], Thomas L. Richie[2], Stephen L. Hoffman[2], Peter G. Kremsner[1]

[1]University of Tuebingen, Tuebingen, Germany, [2]Sanaria Inc., Rockville, MD, United States

Exposure to 12-15 *Plasmodium falciparum* (Pf)-infected mosquitoes, administered three times at monthly intervals under continuous chemoprophylaxis with chloroquine is highly efficacious in preventing asexual blood stage infection following subsequent controlled human malaria infection (CHMI) in healthy, adult, malaria-naïve individuals. To translate and expand this immunization strategy, mosquito bites need to be replaced by a pharmaceutical product that can be easily administered and exactly dosed. In addition, the chemoprophylactic regimen needs to be adjusted to the specific requirements of this approach. Our previous study (TUECHMI-001) showed that one mosquito bite corresponds to ~620 intravenously (IV) injected, cryopreserved Pf sporozoites (PfSPZ) produced by Sanaria Inc. Here, we report first results on the safety, tolerability, immunogenicity and preliminary protective efficacy of escalating doses of Sanaria's PfSPZ (PfSPZ Challenge) under chemoprophylaxis (PfSPZ-CVac approach) with chloroquine. During immunization, PfSPZ were injected by direct venous inoculation (DVI) three times at 4-week intervals. Volunteers receive staggered doses of 3,200 (Group A), 12,800 (Group B) or 51,200 (Group C) PfSPZ per injection, corresponding to approximately 5, 20 or 80 mosquito bites. In every group, 9 volunteers received PfSPZ and 5 placebo, while all received 10 mg/kg chloroquine 2 days before the first injection, followed by 5 mg/kg every week for a total of 10 doses. To assess efficacy of the immunization regimen, CHMI will be done 8 weeks after completion of chemoprophylaxis by DVI of 3,200 PfSPZ Challenge. Subsequently, a PfSPZ-CVac dose that shows at least 75% efficacy, good safety and tolerability will be tested using an experimental ultra-short chemoprophylaxis with azithromycin and chloroquine. Here, 2 g extended-release azithromycin will be given on the day of each PfSPZ injection followed by 10 mg/kg chloroquine 5 days later. Complete results for the first two dose groups will be presented together with safety, tolerability and preliminary immunogenicity data of the highest dose (51,200 PfSPZ).

1609

LONGITUDINAL ANALYSIS OF HUMORAL AND CELLULAR IMMUNITY FOLLOWING VACCINATION WITH AN ATTENUATED *PLASMODIUM FALCIPARUM* SPOROZOITE VACCINE

Andrew Ishizuka[1], Kailan-Sierra Davidson[1], Kim Lee Sim[2], Stephen Hoffman[2], **Robert A. Seder**[1]

[1]Vaccine Research Center, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, [2]Sanaria Inc., Rockville, MD, United States

A highly effective and durable vaccine for preventing *Plasmodium falciparum* (Pf) malaria infection is a critical need for preventing the substantial morbidity and mortality incurred by this infection. Pf sporozoites (PfSPZ) administered by mosquito bites are the only immunogens shown to induce high-level sterilizing protection (>80%) in humans. We previously reported that attenuated, aseptic, purified, cryopreserved PfSPZ (PfSPZ Vaccine) administered 4 or 5 times intravenously (IV) conferred high-level protection in humans in a dose-dependent manner. Moreover, initial analysis of humoral and cellular immunity showed that there was a dose-dependent increase in CSP antibody titer, functional inhibition of sporozoite invasion *in vitro* and the frequency of sporozoite specific IFN-g producing CD4 and CD8+ T cell responses. Here, we substantially expanded this analysis and performed a longitudinal assessment of antibody and cellular responses through the course of vaccination and after controlled human malaria ~ 3 weeks after the final immunization and ~ 5 months later. For T cell assessment, multi-parameter flow cytometry with two recently developed 16-color panels as used to assess the magnitude, phenotype and quality of sporozoite specific T cell responses. Together, these data provide insights into the durability of immunity and protection after vaccination with the PfSPZ Vaccine and will guide ongoing and future studies to define the mechanistic correlates of protection.

1610

CLINICAL DEVELOPMENT OF THE PFSPZ VACCINE TO PROTECT THE WARFIGHTER FROM MALARIA

Judith E. Epstein[1], Kristopher Paolino[2], Sharina Reyes[1], Robin Nielsen[2], Alexandra Singer[1], Martha Sedegah[1], Richard Ruck[2], Carlos Vasquez[1], Elizabeth Saverino[3], Silas Davidson[2], Peter F. Billingsley[3], Anita Manoj[3], Sumana Chakravarty[3], Kavita Tewari[3], Eric James[3], Anusha Gunasekera[3], Adam Richman[3], Matthew Stewart[3], Deborah Padilla[3], Tooba Murshedkar[3], Thomas L. Richie[3], B. Kim Lee Sim[3], Eileen Villasante[1], Stephen L. Hoffman[3]

[1]Naval Medical Research Center, Silver Spring, MD, United States, [2]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [3]Sanaria Inc., Rockville, MD, United States

A vaccine to prevent malaria for use by the Department of Defense (DoD) must provide sustained sterile protection against infection. The Sanaria® PfSPZ Vaccine has been developed to address this need. It is composed of aseptic, purified, cryopreserved, vialed PfSPZ manufactured in compliance with all regulatory standards. In a recent clinical trial, PfSPZ Vaccine protected 6/6 (100%) subjects against controlled human malaria infection (CHMI) at the highest dosage regimen administered (5 intravenous doses of 1.35x10^5 PfSPZ), and there was a dose response in regard to antibody and T cell responses. The protective regimen was safe and well tolerated, indicating that further dose escalation could be undertaken. Since completion of that study, 6 additional PfSPZ Vaccine trials have been or will be initiated, designed to optimize dose size, dose interval, number of doses and duration of protection. This study, conducted by the DoD, addresses the following questions: (1) Is PfSPZ Vaccine safe and tolerable administered by direct venous inoculation? (2) Can 5 doses of 2.7x10^5 PfSPZ provide protection 3 weeks (short term) after immunization against CHMI carrying a heterologous Pf strain? (3) Can 5 doses provide protection when subjects undergo a second CHMI at 24 weeks (long term) with homologous and heterologous Pf parasites? 4) Can the number of doses required to provide short term and long term homologous protection be reduced (to 3 doses of 4.5x10^5 PfSPZ)? We will present safety and immunogenicity results for both the 3 and 5 dose regimens. Additionally, we will present our plans to conduct late Phase 2 and Phase 3 trials supporting a BLA for licensure in adults. The potential for worldwide benefit results from the fact that DoD requirements - excellent safety and tolerability, efficient administration, and sterile protection lasting for at least six months - are characteristics that equally support deployment to malaria endemic areas to prevent disease and death and to promote campaigns aiming to halt malaria transmission and eliminate the disease from defined geographical areas.

1611

MAN VERSUS MOSQUITO: HOW VECTOR-BORNE PATHOGENS MOVE AROUND THE WORLD

Michael Johansson[1], Andrew Tatum[2]

[1]Centers for Disease Control and Prevention, San Juan, PR, United States, [2]University of Southampton, Highfield, United Kingdom

Vector-borne pathogens, such as malaria and dengue, are major causes of morbidity and mortality, worldwide. These pathogens often move between endemic areas and from endemic to non-endemic areas via airplanes, which can rapidly transport infected humans and vectors over long distances. Upon introduction, either the human or vector may initiate new transmission cycles in other locations under the right ecological conditions. While numerous countries have established regulations for disinsection of airplanes arriving from certain locales, it has not been clear whether this significantly reduces transmission risk. We developed branching process models to assess the probability of traveling humans and vectors initiating transmission resulting in at least one local human infection in a new location. For humans, this depends on the probability of an infected person traveling, infecting a mosquito, and that mosquito infecting a human, as well as several constituent processes. For mosquitos, it is the probability of an infected mosquito traveling and infecting a human, again with constituent processes. We assessed these models for *Plasmodium falciparum*, a causative agent of malaria. For a plane moving from a highly endemic area to another area highly suitable for *P. falciparum* transmission, the probability of introduction of *P. falciparum* by a human is approximately 100%. However, for a mosquito it is less than 0.1%. Analysis of the sequence of events leading to introduction makes it clear that mosquitoes have lower probabilities of travel, infection, and further transmission compared to humans for whom the risk of pathogen introduction is many times larger. While controlling the transportation of mosquitoes may be critical for avoiding the introduction of vector or pest species, our model indicates that it has little benefit for vector-borne pathogens. Given the ever-increasing volume of travel, it is critical to develop new ways to reduce the risk of pathogen spread by infected humans.

1612

DURABILITY OF POLYESTER-BASED LONG-LASTING INSECTICIDAL NETS IN THREE GEOGRAPHICAL ZONES OF NIGERIA - A THREE YEAR FOLLOW-UP OF NETS DISTRIBUTED THROUGH CAMPAIGNS

Albert Kilian[1], Emmanuel Obi[2], Richmond A. Selby[3], Hannah Koenker[4], Matt Lynch[4]

[1]Tropical Health LLP, Montagut, Spain, [2]Malaria Consortium, Abuja, Nigeria, [3]Malaria Consortium, Kampala, Uganda, [4]Centers for Communication Programmes, Baltimore, MD, United States

The average survival of Long-lasting Insecticidal Nets (LLIN) has increasingly become of interest to malaria program managers and the international community as this measure of durability will determine the frequency of LLIN replacements at household level and the cost of sustaining universal coverage. With the recent publication of a WHO-recommended method

to estimate net survival, comparative analyses from different areas or for different brands have now become possible. Following the mass distribution campaigns of 2010/11 in Nigeria annual follow-up surveys to measure attrition and physical integrity of campaign LLIN (polyester, 100 Denier) were undertaken in three locations of Nigeria representing different eco-geographic and climatic zones: Shinkafi district in Zamfara State in the northern dry-savannah, Toto district in Nasarawa State in the central guinea-savannah and Abi district in the rain-forest area of Cross River State. In each district a population representative sample was drawn using a 20 cluster sampling design and in each selected community 15 households that had received nets from the campaign were included in the interview and net assessment. In the questionnaire reasons for any loss of campaign nets were explored as well as attitudes and practices towards net care and repair. The assessment of physical integrity of the nets was done according to WHO recommendations and the proportionate Hole Index used to evaluate the outcome for each net. In the first round of surveys a total of 900 households were sampled and 1,571 campaign nets assessed while for the second round the figures were 896 and 1,367 respectively. Two years after the campaign the survival of LLIN varied considerably between locations with 69.6% (95% CI 62.7-75.7) still in serviceable condition in Nasarawa State, 81.4% (75.0-86.5) in Zamfara and 89.4% (84.2-93.5) in Cross River State. The final round of data collections is currently ongoing and will be presented together with estimates of median LLIN survival. Reasons for differences by location will be explored and discussed.

1613

MODELING THE EFFECTS OF TRANSNATIONAL MIGRATION ON PUBLIC HEALTH POLICIES IN SOUTHEAST ASIA: AN INVESTIGATION INTO THE IMPACT ON ELIMINATION STRATEGIES

Amruta Dixit[1], Ming-Chieh Lee[1], Edward Wenger[2], Guiyun Yan[1]

[1]University of California Irvine, Irvine, CA, United States, [2]Institute for Disease Modeling, Seattle, WA, United States

Human migration plays an important role in the spread of infectious diseases. In order to determine the impact of human movement between areas of unequal malaria endemicities on malaria transmission and the resulting control and elimination strategies, we need to improve our understanding of: the migration patterns present in the study areas of interest, the malaria acquisition risk borne by the migrants, and the impact of migration on malaria transmission. We hypothesized that persistent and unmonitored flow of people between porous borders between areas of unequal transmission, presents a significant challenge to the elimination of malaria in the area of low disease transmission. The present study aims to provide a quantitative assessment of malaria risks due to transnational migration and to evaluate subsequent intervention strategies for malaria control and elimination. Within the EMOD framework developed by the Institute for Disease Modeling, we have constructed simulations with two geographically-connected populations in order to model the impact of varying degrees of human migration rates and intervention methods on malaria transmission. Our preliminary simulation results suggest that the proportion of humans infected was markedly different between the various rates of human migration. We will continue to develop increasingly complex simulations that explore both human and vector-based interventions such as the administration of primaquine and the application of long-lasting microbial larvicides. The simulations results can be used to consider various feasible pathways to sustained local elimination as a function of cross-border migration rates. This approach has the ability to make a timely and significant contribution to public health by filling a critical gap in our knowledge of human migration patterns, especially in Southeast Asia. The utility of these data and this novel modeling tool is multidisciplinary and has the potential to inform sophisticated models in other research fields.

1614

ANALYSIS OF DETERMINANTS OF INSECTICIDE TREATED NETS (ITNS) USE AMONG CHILDREN UNDER FIVE YEARS USING LOGISTIC REGRESSION

Wahjib Mohammed[1], Anthony J. Turkson[2], David Y. Mensah[1]

[1]National Malaria Control Programme, Accra, Ghana, [2]Takoradi Polytechnic, Accra, Ghana

Malaria causes between three hundred and fifty (350) and five hundred (500) million clinical episodes and over one million deaths annually. Children and pregnant women are the most vulnerable group and most endangered by the disease. Insecticide-Treated bednets (ITNs) range among the most effective measures of malaria prophylaxis, yet its implementation level in sub-Saharan Africa is still low. The goal of this study was to assess factors influencing the use of ITNs by children under five years in Ghana. A cross-sectional study was conducted using pre-tested, interviewer-administered questionnaires. Possible factors were measured using five hundred (500) mothers or guardians of children under five years, in twenty three (23) communities in the Asamankese sub-municipality in the West Akim District in the Eastern Region of Ghana. Logistic regression was used to assess the influence of five possible factors on ITNs use. In order of importance in determining one's use of LLIN, 'Sleeping area allowing for the use of Long Lasting Insecticides Net (LLIN)' was the most important factor influencing the use of ITNs by children under five years in Ghana, followed by 'Household's expected monthly income', then 'Mother's SHS level of education' compared to 'none', while the least was 'Number of children under five years'. The study also revealed that 86.2% of the participants owned ITNs out of which 92.81% used it the night before the study. The study revealed that there is a relationship between influencing factors and use of LLINs. To improve ITNs usage, there should be continuous distribution of LLINs and Insecticide Treated Materials (ITMs), such as insecticide treated curtains for doors and windows. This should be heavily supported by education and Behavior Change Communication (BCC) via radio, TV, and other media especially on the hanging techniques and need to provide adequate space for sleeping area.

1615

EVALUATING DIFFERENCES IN HOUSEHOLD INSECTICIDE-TREATED BEDNETS (ITN) OWNERSHIP BETWEEN UGANDA AND ZIMBABWE DURING 2005-2011: AN ECONOMETRIC APPROACH

Yesim Tozan[1], Selver Derya Uysal[2]

[1]New York University, New York, NY, United States, [2]Institut für Höhere Studien, Vienna, Austria

The rapid scale-up of insecticide-treated bednets (ITNs) in African countries have received heightened attention with the availability of significantly greater resources for national malaria control efforts, particularly after 2005. In this study, we investigate the mean differences in household ITN ownership in Uganda and Zimbabwe for the years 2005 and 2011, using DHS and GPS data and MARA malaria endemicity maps and econometric methods. The probability of owning an ITN in Uganda was 12.8% higher than in Zimbabwe in 2005. Although ITN ownership increased steadily in both countries, the difference in the probability of owning an ITN widened significantly and became 33.5% over this period. The Blinder-Oaxaca technique can be used to study the mean outcome differences between two groups (in our case two countries). Using this technique, we divide the ITN ownership differential between two countries into a part that is "explained part" by group differences in ITN ownership determinants, such as household characteristics and malaria risk, and a residual part that cannot be accounted for by such differences in ITN ownership determinants. This "unexplained" part captures the effects of group differences in unobserved predictors of ITN ownership. Our preliminary results showed that the larger fraction of the increase in the ITN ownership differential in this period was due to the unexplained part. To understand

this result we investigated what happened in these countries in terms of malaria control efforts between 2005 and 2011. Published literature points to a significant difference in malaria financing in these countries, yet the earmarked proportion of funding for increasing the ownership and use of ITNs is usually high in all countries. Next we will study to what extent malaria control efforts in these two countries affected ITN usage and all-cause child mortality rates and potentially extend our analysis to include other African countries.

1616

UNDERSTANDING INTRA-HOUSEHOLD DECISION MAKING FOR INSECTICIDE-TREATED MOSQUITO NET ALLOCATION IN UGANDA

Emily E. Ricotta, Steve A. Harvey, Hannah Koenker, Matthew Lynch

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

Intra-household allocation of insecticide-treated mosquito nets (ITNs) is an important concern for malaria prevention programs. Behavior change communication campaigns stress the need to prioritize children under five and pregnant women for net distribution and use, as these two groups are the most vulnerable to malaria infection. This study utilized a pile sort activity to help understand net allocation decision making in Luwero and Nebbi, two Ugandan districts. Sixty-four respondents, half men and half women, were asked to assign 10 individuals ranging in age from infant to older adult and including adults and children of both sexes as well as one visibly pregnant woman to one of four beds. One bed had a new net, one a slightly used net with a few holes, one an old net with large holes, and the last with no net. After sorting, respondents were asked why they allocated each individual as they had. The number of times a household member was placed under each net was evaluated, and a hierarchical cluster analysis was completed to determine which household members were typically grouped together. Responses were compered between male and female, urban and rural, and district of residence. Results demonstrate that net allocation differs by gender, rural/urban, and district, however the pregnant woman and baby were always clustered together and were given the best net greater than 50 and 60 percent of the time, respectively. Children ages 5-14 were also typically clustered together and given the best or second-best net. Young adults (around age 25), older adults, and elders were given the worst net or no net a majority of the time. Reasons given for these placements varied, but common themes include the baby and pregnant women being the most vulnerable to malaria, the older children being able to help repair nets, and the heads of household and young adults being robust enough to not succumb to malaria infection and having sufficient resources to purchase additional nets. The general consensus was that this household did not have enough nets, and that the family should strive to provide nets for all of their household members. This exercise supports current behavior change campaign messaging, demonstrating that, at least in their reported behavior, people are likely to prioritize vulnerable populations when it comes to net use.

1617

REDUCTION IN DISPARITY OF INSECTICIDE-TREATED NETS OWNERSHIP AND USE AMONG SOCIOECONOMIC GROUPS AFTER SCALE UP IN UGANDA

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

ICF International, Rockville, MD, United States

The increase in funding for malaria control in the past decade resulted in an increase in Insecticide-Treated Nets (ITNs) ownership and use in many countries in sub-Saharan Africa, particularly in Uganda. However, with the shift in programmatic focus from target groups to universal coverage there is a need to ensure equal access and use of ITNs for all sub-populations regardless of their socioeconomic status. This study assessed change in disparity in ITN ownership and use among different socioeconomic groups in Uganda between 2006 and 2011. The authors used Lorenz Concentration Curve and Index (C-Index) to assess equity in household ITN ownership and use among children under five between wealth quintiles separately in 2006 (Demographic and Health Survey data) and 2011(Malaria Indicator Survey data). C-Index values range between -1 to 1, a value of 0 suggests no difference in ownership and use among different socioeconomic groups. Household ownership of at least one ITN rose significantly from 16% (2006) to 60% (2011). Similarly, ITN use among children under five was very low (10%) in 2006 and increased substantially to 47% in 2011. The increase in ITN ownership was associated with significant reduction in inequity among wealth quintiles (C-Index 0.11, 95% CI: 0.08;0.34) in 2006 versus 0.02, 95% CI: 0.01;0.04 in 2011). Similarly the disparity in use of ITN use among children under five from different wealth quintiles greatly reduced from 2006 (C-Index: 0.04, 95% CI:-0.10;0.19) to 2011 (C-Index: 0.01, 95% CI:-0.04;0.06). This achievement is probably due to the shift to universal coverage in 2009 which led to free mass distribution campaigns of Long-lasting Insecticidal Nets (LLINs), with 7.2 million LLINs distributed by 2010. This achievement in parity between wealth quintiles should be sustained; however, efforts are needed to further increase overall ITN ownership coverage and use in Uganda. This is achievable through additional free mass campaign distribution combined with traditional distribution channels.

1618

ASSESSING THE CONTRIBUTION OF MALARIA CONTROL INTERVENTIONS ON REDUCTIONS IN ALL-CAUSE UNDER-5 MORTALITY IN ZAMBIA

Katherine E. Colson[1], Laura Dwyer-Lindgren[1], Nancy Fullman[1], Tom Achoki[2], Matthew Schneider[3], Peter Hangoma[4], Marie Ng[1], Felix Masiye[5], Emmanuela Gakidou[1]

[1]Institute for Health Metrics and Evaluation, Seattle, WA, United States, [2]Ministry of Health of Botswana, Gaborone, Botswana, [3]United States Agency for International Development, Washington, DC, United States, [4]Department of Economics, University of Bergen, Bergen, Norway, [5]Department of Economics, University of Zambia, Lusaka, Zambia

Between 2000 and 2010, Zambia rapidly scaled-up malaria control interventions such as insecticide-treated nets and indoor residual spraying. At the same time, Zambia recorded substantial declines in under-five mortality. Zambia's experience is heralded as a malaria-control success story, but it is unclear whether the expansion of malaria control contributed to improved childhood survival beyond the impacts of other child health interventions. To quantify the impact of malaria control efforts in Zambia, we estimated trends for all-cause under-five mortality and a range of child and maternal health interventions at the subnational level. We quantified the reduction in child mortality associated with malaria control while taking into account trends in other key interventions as well as socio-demographic, health system, and environmental factors across districts. Our estimation methods included generalized linear models and functional data analysis and were validated with cross-validation and simulation techniques. We found that the bivariate relationship between malaria control and child mortality was strong and significant, but this relationship was attenuated when other factors were considered. Several other child health interventions also scaled up dramatically during the same time period, including pentavalent immunization, prevention of mother-to-child-transmission of HIV/AIDS, exclusive breastfeeding, and nutrition programs. Because of this simultaneous expansion, it was statistically infeasible to isolate the effects of malaria control efforts. In the absence of the combined scale-up of these interventions between 2000 and 2010, we estimated that child mortality would have been 11% higher in 2010. The scale up of these interventions accelerated declines in mortality by 1% each year. Our findings emphasize the importance of constructing a comprehensive landscape of the drivers of progress in child mortality. A greater quantity of high-quality and localized data is critical for evaluating the independent impact of each intervention on childhood survival.

1619

INCREASING ROLE OF *ANOPHELES FUNESTUS* AND *AN. ARABIENSIS* IN MALARIA TRANSMISSION IN THE KILOMBERO VALLEY, TANZANIA

Dickson W. Lwetoijera[1], Caroline Harris[2], Samson Kiware[1], Stefan Dongus[3], Gregor Devine[4], Philip McCall[5], Silas Majambere[1]

[1]Ifakara Health Institute, Ifakara, Morogoro, United Republic of Tanzania, [2]Lancaster University, Lancaster, United Kingdom, [3]Swiss Tropical and Public Health Institute, Basel, Switzerland, [4]QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia, [5]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

This longitudinal study demonstrates the trends in malaria vector dynamics and their relative contribution to malaria transmission in hyper-endemic transmission settings in Tanzania. The study was conducted in two villages within the Kilombero valley, in rural Tanzania for five consecutive years (2008 - 2012). 72 houses were selected per village and each house was sampled for mosquitoes monthly using a CDC light trap. Collected mosquitoes were assessed for species identity and sporozoite infection status using PCR and ELISA respectively. *Anopheles funestus* susceptibility to insecticides was assessed using WHO guidelines. A total of 100,810 malaria vectors were collected, of which 76% were *An. gambiae* s. l. and 24% were *An. funestus*. Of all *An. funestus* samples that amplified with PCR (n = 2,737), 97% were *An. funestus* s.s., 2% were An. rivorulum and 1% An. leesoni. Whereas for *An. gambiae* s.l. (n = 8,117), 93% were *An. arabiensis* and 7% were *An. gambiae* s.s. The proportion of *An. gambiae* s.s. identified by PCR (2,924) declined from 0.2% in the year 2008 to undetectable levels in 2012. *An. arabiensis* dominated the wet season whereas *An. funestus* dominated the dry season. Malaria transmission intensity significantly decreased from an EIR of 78.14 infectious bites/person/year in 2008 to 35ib/p/yr in 2011 but rebounded to 226 ib/p/yr in 2012 coinciding with an increased role of *An. funestus* in malaria transmission. Insecticide susceptibility tests indicated full susceptibility of *An. funestus* to deltamethrin (100% mortality), reduced susceptibility to dieldrin (95%), permethrin (93%), and confirmed resistance to DDT (86%). Similar findings were also recorded for An. arabiensis, in separate study in same area. The results indicate the continuing role of *An. arabiensis* and the increasing importance of *An. funestus* in malaria transmission. These findings call for complementary vector control and surveillance tools that target these specific vector species, their behaviour and their ecology and an insecticide resistance management plan to preserve the efficacy of LLINs.

1620

DEVELOPMENT OF AN *IN VITRO* TRANSMISSION BLOCKING (ITB) ASSAY AGAINST *PLASMODIUM FALCIPARUM* WITHOUT THE USE OF MOSQUITOES

Abraham G. Eappen, Tao Li, Meghan Marquette, Peter F. Billingsley, Adam Richman, B. K. Sim, Stephen L. Hoffman

Sanaria Inc., Rockville, MD, United States

Major efforts are underway aimed at developing malaria vaccines that can be used for elimination of *Plasmodium falciparum* by preventing the development of the parasite within the mosquito and thereby preventing transmission of the parasite to humans. To assess candidate vaccines, antibodies are induced by immunization of animals and humans, and assessed for their transmission blocking activity against sexual erythrocytic and mosquito stage parasites in a standard membrane-feeding assay (SMFA). These SMFAs are biologically relevant, but highly consumptive of personnel time and resources, require a mosquito colony and are not easily adaptable to high through-put. Thus, it is difficult, time consuming and expensive to assess large numbers of candidate anti-sera in SMFAs. Sanaria's technology platform generates live, aseptic, purified, cryopreserved *P. falciparum* sporozoites (PfSPZ) that can be administered as a highly protective malaria vaccine. PfSPZ are produced using *Anopheles stephensi* mosquitoes as bioreactors. Sanaria, in its quest for developing PfSPZ-based products without the use of mosquitoes, developed technology for the *in vitro* production of Pf oocysts. We have optimized gametocyte culture conditions that are optimal for the *in vitro* production of ookinetes and oocysts, established culture conditions to reproducibly produce and quantify 3 and 7 to 8 day oocysts, and demonstrated that *in vitro* produced oocysts are similar in size and morphology to mosquito produced oocysts. These developments have made it possible to assess the transmission blocking activities of candidate vaccines in an *in vitro* transmission blocking (iTB) assay without the need for mosquitoes. Preliminary data indicate correspondence between the results of SMFA and iTB assays. The transmission blocking activity of antibodies against Pfs25 and Pfs48/45 in SMFA and iTB assay will be presented.

1621

IDENTIFICATION OF A NOVEL *PLASMODIUM FALCIPARUM* HEAT SHOCK PROTEIN 70 (HSP70Z), ALSO IDENTIFIED AS CG4, AS A TRANSMISSION BLOCKING VACCINE CANDIDATE

Mark DeCotiis, Joan Aebig, Buddhadeb Mallik, Nikiah Dawson, Olga Muratova, Karine Reiter, Martin Burkhardt, Vu Nguyen, Patrick E. Duffy, Nicholas J. MacDonald, **David L. Narum**

National Institutes of Health, Rockville, MD, United States

A malaria transmission blocking vaccine (TBV) is critical to achieve the goal of malaria elimination in some areas. To date, only one sexual stage protein, Pfs25, has been evaluated in humans and a second sexual stage protein, Pfs230, will begin human testing soon. Other sexual stage proteins have failed to reach the clinic due to an inability to produce them at pilot-scale following cGMP. In order to expand the number of TBV candidates, a panel of monoclonal antibodies (mAbs) was produced against *Plasmodium falciparum* macrogametes to identify novel surface proteins that, when targeted by antibodies could interfere with parasite development in the mosquito midgut. Here we report on the evaluation of one mAb, identified as 1C7, which recognized a heat shock protein (HSP), identified as HSP70z or Cg4, by Western blot of a sexual stage parasite lysate and by pull-down studies using LC/MS/MS techniques. HSP70z is expressed in asexual and sexual stage parasites with a molecular mass of approximately 100 kDa. In macrogametes, HSP70z may be localized on the macrogamete cell surface by a live immunofluorescence assay. Most importantly, 1C7 blocked *P. falciparum* transmission in mosquitoes, with similar activity to that of a Pfs230 domain 1 specific mAb using an *ex vivo* membrane feeding assay. A recombinant form of HSP70z (named rHSP70z) was produced in *Pichia pastoris* that was comprised of approximately 20% of the native protein which was recognized by 1C7 in Western blots. rHSP70z specific IgG purified from sera of immunized rabbits failed to block parasite transmission. In a competition ELISA, rHSP70z specific rabbit antibodies failed to compete for 1C7 binding to rHSP70z, likely explaining the lack of transmission blocking activity. Currently, the 1C7 epitope is being mapped to evaluate whether a synthetic peptide mimicking the 1C7 epitope will induce transmission blocking antibodies.

1622

IMPACT OF INDOOR RESIDUAL SPRAYING ON ENTOMOLOGICAL INDICES OF MALARIA TRANSMISSION IN THE BUNKPURUGU-YUNYOO DISTRICT IN THE NORTHERN SAVANNAH ZONE OF GHANA

Samuel Dadzie[1], **Sylvester Coleman**[2], Peter Mumba[2], Aklilu Seyoum[2], Philip Ricks[3], Daniel Szumlas[4], Paul Psychas[5], Jacob Williams[6], Maxwell Appawu[1], Daniel Boakye[1]

[1]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [2]Ghana Africa Indoor Residual Spraying Project, Abt Associates, Accra, Ghana, [3]Malaria Branch, Division of Parasitic Diseases and Malaria, Atlanta, GA, United States, [4]Centers for Disease Control and Prevention, Atlanta, GA, United States, [5]University of Florida, Gainesville, FL, United States, [6]Research Triangle International, Washington, DC, United States

Malaria remains a major public health problem in Ghana, especially in the northern savannah zone. This study was conducted to evaluate the impact of U.S President's Malaria Initiative (PMI) and the Ghana Health Services Indoor Residual Spraying (IRS) program on malaria transmission in the Bunkpurugu-Yunyoo District in northern Ghana. In 2011 and 2012, the district was sprayed with a pyrethroid, alphacypermethrin, at an application rate of 25mg/m^2. In 2013, an organophosphate, pirimiphos-methyl at a rate of 1g/m^2), was used based on declining susceptibility of local vectors to pyrethroids. Indoor resting densities (IRD), parity, sporozoite rate and entomological inoculation rates (EIR) of the local vector species were monitored through pre- and post-IRS monthly human landing and pyrethrum spray collections. The IRD of *Anopheles gambiae* s.l. (the predominant vector species, 99.2% of all *Anopheles* collected) was reduced from a mean of 2.91 mosquitoes/room recorded from the baseline surveys to 2.10 mosquitoes/room (27.7% reduction) in 2012 after spraying with alphacypermethrin. In 2013, the mean IRD of *An. gambiae* s.l. was further reduced to 0.22 mosquitoes/room, representing 89.2% decline compared to 2012. In 2012 there was a non-significant reduction (p=0.289) in the mean parity rate for *An. gambiae* s.l. from 75% to 43% (57% reduction). Spraying with Actellic in 2013, resulted in 67% reduction in parity rate from 43% to 32% (p = 0.130). A comparison of the pre and post-IRS EIRs also revealed a significant (p<0.05) reduction, from 0.35 infective bites/man/night (ib/m/n) in 2011 to 0.021 ib/m/n in 2012. In 2013, there was slight decrease in EIR to 0.018 ib/m/n after spraying with Actellic 300CS that year. The results show that the IRS operations resulted in reduction in key entomologic indicators. IRS with pirimiphos-methyl had the greatest impact on indoor resting densities and parity rates of *An. gambiae* s.l. but not EIRs. The PMI funded IRS program after three years contributed to 94.8% reduction in malaria transmission in the study area.

1623

SCALING UP OF INSECTICIDE-TREATED NETS (ITN) OWNERSHIP AND USE IN MOZAMBIQUE: HAS THE SCALE-UP BEEN EQUITABLE?

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

ICF International, Rockville, MD, United States

Insecticide-treated nets (ITNs) are effective tools for malaria prevention and can significantly reduce morbidity and mortality due to malaria, especially among children under five in endemic areas. ITN ownership and use have rapidly increased in Mozambique, due to scaling up of ITN distribution efforts within the country over the past seven years, which have aimed to achieve universal coverage. The percent of households that owned at least one ITN rose by more than three-fold from 15.7% in 2007 to 51.4% in 2011. ITN access, defined as one ITN for every two people in the household also rose significantly, from 9.4% in 2007 to 37.0% in 2011. Similarly during this time, ITN use among children under five and pregnant women increased significantly, from 6.7% to 35.7% and from 7.3% to 34.3%, respectively. With the aim of achieving universal coverage, it is important to also assess distribution across subgroups, particularly groups from different socio-economic status. This analysis used Lorenz concentration curves and indices to assess the equity of ITN household ownership, access and use in Mozambique, using data from the 2007 Malaria Indicator Survey and 2011 Demographic Health Survey. Concentration Index (C-Index) values range between -1 and 1, with a value of 0 representing perfect equality. From 2007 to 2011, equity in ITN household ownership (C-Index: 0.06 in 2007 and 0.04 in 2011), ITN access (C-Index: 0.12 in 2007 and 0.09 in 2011), and ITN use among children under five (C-Index: -0.04 in 2007 and 0.03 in 2011) showed slight improvements, while equity in ITN use among pregnant women remained the same (C-Index: .06 for both years). It is important to highlight that while improvements where shown, equity in ITN household ownership, access and ITN use among children under five, all were fairly equitable in 2007. The results demonstrate that ITN scale-up efforts have been successful as well as equitable across the population, however further improvements in access and ITN coverage are still needed to reach targets.

1624

DIFFERENCES IN DEET AND PICARIDIN SENSITIVITY BETWEEN SOUTHEAST ASIAN VECTORS OF MALARIA AND ARBOVIRUSES, RESULTS OF A FIELD EVALUATION OF TOPICAL REPELLENTS

Lies Durnez[1], Mao Sokny[2], Karel Van Roey[1], Vincent Sluydts[1], Siv Sovannaroath[2], Tho Sochantha[2], Marc Coosemans[1]

[1]Institute of Tropical Medicine, Antwerp, Belgium, [2]National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia

Scaling up of insecticide treated nets has contributed significantly to a substantial malaria decline. However, some malaria vectors, and most vectors of arboviruses, bite outdoors and in the early evening. Therefore, topically applied insect repellents may provide a crucial additional protection against mosquito-borne pathogens. Among topical repellents, DEET is the most commonly used, followed by others such as PMD or picaridin. A study was carried out in Cambodia to determine the entomological efficacy of DEET and picaridin repellents on wild populations of several mosquito genera, including vectors of arboviruses (*Aedes aegypti* and *Ae. albopictus*) and malaria (*Anopheles dirus*, *An. minimus*, *An. maculatus* and *An. barbirostris*). During 230 survey days in two consecutive years, the lower limbs of 5 persons were treated with repellents ('DEET 20%', 'picaridin 20%', or 'picaridin 10%') or ethanol (2 negative controls), followed by mosquito collections on the treated limbs during 5 consecutive hours. The treatments were grouped following a 5x5x5 Graeco-latin square to equalize the effects of treatment days, collection sites, and test persons. Protection rates were high (91-99.2%), with significant differences between treatments, genera, and species. For malaria vectors, 'DEET 20%' performed better than 'picaridin 20%' or 'picaridin 10%'. The protection rate against *An. barbirostris* was significantly lower as compared to the other vectors, especially for the picaridin repellents. As malaria endemic areas often differ in their vector species composition, this heterogeneity in repellent sensitivity between vector species might result in a geographically heterogeneous epidemiological impact of repellent use for malaria control.

1625

MOLECULAR AND ENVIRONMENTAL INTERACTIONS IN *ANOPHELES GAMBIAE* INFLUENCE BOTH REPRODUCTIVE CAPACITY AND *PLASMODIUM* DEVELOPMENT

Perrine Marcenac[1], Francesco Baldini[2], Nicola Segata[3], Abdoulaye Diabate[4], Flaminia Catteruccia[1]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Università di Perugia, Terni, Italy, [3]University of Trento, Trento, Italy, [4]Institut de Recherche en Sciences de la Sante/Centre Muraz, Bobo-Dioulasso, Burkina Faso

Reproduction in the major malaria vector *Anopheles gambiae* is influenced by a number of molecular and environmental factors. Male-female

molecular interactions following mating are important determinants of fertility and fecundity. At the same time, an increasing amount of evidence points to reproductive processes playing an important role in *Plasmodium* parasite development. Male transfer of the steroid hormone 20-hydroxy-ecdysone (20E) during mating activates the transcription of a *Mating-Induced Stimulator of Oogenesis* (*MISO*) gene that transduces the mating signal into an increase in egg development. Silencing *MISO* by RNA interference in laboratory colonies reduces egg development to levels observed in virgin females. This phenotype is caused by reduced expression of yolk protein precursors (YPPs) after *MISO* silencing, impairing lipid accumulation in the oocyte. Previous research shows that the same YPPs essential for lipid accumulation in the developing mosquito egg help parasites escape the immune system. We have found evidence that MISO depletion decreases *Plasmodium* infection in *A. gambiae*, further reconstructing the molecular pathways linking egg development and *Plasmodium* infection. Functional studies performed in field *A. gambiae* show that in natural populations *MISO* is essential for egg development, as its silencing abolishes oogenesis after blood feeding. These results suggest that in natural mosquito populations MISO is a key switch that directs resources derived from the blood meal towards oogenesis only in mated females. Besides molecular factors, environmental factors also affect *A. gambiae* reproductive biology in natural populations. Sequencing analysis of the microbiota from reproductive tissues of wild *A. gambiae* demonstrates enrichment of bacteria in specific villages and mating swarms, which may impact reproductive success and isolation. In addition we have identified bacteria in reproductive tissues that have been previously shown to impact *Plasmodium* survival. We are currently determining the effects of these bacteria on reproductive biology and mosquito fitness, two factors relevant for malaria transmission. By elucidating the different molecular and environmental interactions regulating reproduction and parasite development in *A. gambiae*, we hope to develop novel targets for vector control.

1626

HETEROGENEITY OF HUMAN AND MOSQUITO BEHAVIOR IN RELATION TO OUTDOOR AND EARLY MALARIA TRANSMISSION IN SOUTHEAST ASIA

Charlotte Gryseels[1], Lies Durnez[1], Sambunny Uk[2], Vincent Sluydts[1], Tho Sochantha[2], Somony Heng[2], Karel Van Roey[1], Lydie Canier[3], Didier Menard[3], Sokny Mao[2], Saorin Kim[3], Marc Coosemans[1], Koen Peeters Grietens[1]

[1]Institute of Tropical Medicine Antwerp, Antwerp, Belgium, [2]National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia, [3]Institut Pasteur du Cambodge, Phnom Penh, Cambodia

Outdoor transmission is one of the key factors for malaria control and elimination in the Greater Mekong Region, including Cambodia, where malaria is now mainly reduced to forested regions inhabited by ethnic minorities and mobile migrants. Tackling outdoor transmission requires a better understanding of the heterogeneity in human and vector behavior during times when people are still active outdoors. Results from a mixed-methods social science study in Ratanakiri, Cambodia, indicate that local ethnic minorities have different socio-cultural characteristics than the majority society targeted by malaria control programs. (i) Mobility caused by a multiple residence system increases exposure to the sylvatic vector *An. dirus*, as during the malaria peak season people usually reside on their farms in the forest. (ii) Open housing blurs the boundary between indoor and outdoor biting. (iii) Differences in sleeping times between villages, farms and during forest activities creates diverse evening biting opportunities, if assumed that people sleep under insecticide-treated nets at night. However, (iv) evening resting is frequently done without nets, and (vi) even night sleeping often occurs under non-impregnated bought nets, which are often torn, making it hard to establish to what extent transmission actually occurs early or outdoors. Results seem to suggest that the evolving interplay of vector and human behavioral heterogeneity maintains malaria hotspots/pops of mainly asymptomatic carriers. Targeting malaria transmission in low transmission settings requires a better understanding of this heterogeneity.

1627

NO PRODUCT? NO PROGRAM: TREATMENT UPTAKE AND AVAILABILITY OF ANTIMALARIAL DRUGS FOR INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN MALAWI

Katherine Wolf[1], Loren Bausell[1], Evance Moyo[2]

[1]United States Agency for International Development | DELIVER PROJECT, Arlington, VA, United States, [2]United States Agency for International Development | DELIVER PROJECT, Lilongwe, Malawi

Throughout Africa, 30 million pregnant women are exposed to malaria each year. Several interventions are central to malaria control efforts, including dispensing sulfadoxine-pyrimethamine (SP) for intermittent preventive treatment in pregnancy (IPTp). While preventing malaria in pregnancy is a key focus of global interventions, trends in using SP to prevent malaria during pregnancy have largely stagnated. Twenty years after adopting IPTp as an official policy, the rates of pregnant women in Malawi who receive at least two doses of SP is only 55 percent. Research has identified factors impeding adherence to IPTp policies: antenatal clinic (ANC) client behavior/attitudes, provider actions/attitudes, and SP availability at facilities. Qualitative data indicate that stockouts significantly impact IPTp coverage. However, only a quantitative analysis will show if there is a relationship between IPTp use and stockouts. For four years, the USAID | DELIVER PROJECT examined trends for three complementary data sources: SP availability at heath facilities using the country's logistics management information system; SP uptake at health facilities using national ANC service statistics; and IPTp coverage in households reported in the Malawi Demographic Health Surveys. Preliminary results show a general decline in SP stockouts between 2010 and 2013. SP stockouts peaked in late 2011 and the first quarter of 2012 (80 percent); low stockout rates were reported during the last half of 2012 and all of 2013 (6 percent). High stockout rates in early 2012 correlate with a sharp drop in SP uptake during the same time (62 to 31 percent). As SP availability improved, beginning in May 2012, the IPT coverage rates also improved. Women attending their first ANC visit after 12 weeks, who received any SP, increased to almost 100 percent by mid-2013. These results show a close relationship between SP availability and the uptake of preventive treatment during pregnancy. Using a mixed-method case study approach, this analysis will explore the impact of SP stockouts on MIP programming efforts.

1628

AN EXTENDED MOLECULAR BARCODE FOR TRACKING *PLASMODIUM FALCIPARUM* PARASITE POPULATIONS

Nicholas K. Baro[1], Rachel Daniels[1], Eli Moss[2], Wesley Wong[1], Daniel L. Hartl[3], Daniel E. Neafsey[2], Sarah K. Volkman[1], Dyann F. Wirth[1]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Broad Institute, Cambridge, MA, United States, [3]Harvard University, Cambridge, MA, United States

The lack of a generalizable approach to malaria control requires a real-time assessment of both epidemiological and parasite population genetics at the local level. The major epidemiological shifts associated with the transition into a malaria-eliminating country are mirrored by changes in the genetic diversity profile of malaria parasite populations. These genetic signatures can be monitored using population genetic - based tools to determine efficacy of malaria intervention efforts prior to measurable changes in disease prevalence. For these reasons, and to further understand the basic biological processes of parasite transmission, we have improved upon an interim panel of neutral, unlinked, single nucleotide polymorphisms (SNPs) that is specifically designed for resolving

individual parasites in highly related parasite populations and allows for quantification of allele balance at polymorphic loci to infer changes in transmission dynamics. The extended molecular barcode includes SNPs exhibiting high minor allele frequency and low divergence (e.g FST) filtered from screening over 500 whole genome sequenced samples representing populations from Africa, South East Asia and South America. The accuracy and sensitivity of Sequenom's Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry genotyping technologies allow for quantitative output that reflects proportions of each allele in multiplexed reactions. The increased number of loci and low dynamic range of the minor allele improve our ability to predict the number of distinct parasite genomes in samples with higher complexity of infection (COI). In silico testing of the extended molecular barcode demonstrates its utility for detecting recent common ancestry among parasites in a sample on a much more cost-effective basis than using variants called with whole genome sequence data. We are exploring the use of these approaches to identify shared regions of the genome that are identical by descent among highly related Senegalese populations and assess COI in high transmission settings.

1629

RECEPTIVITY OF ANOPHELINE MOSQUITOES IN SOUTHERN ZAMBIA: TWO YEARS OF BIONOMIC DATA FROM AN AREA TARGETED FOR MALARIA ELIMINATION

Jennifer C. Stevenson[1], Limonty Simubali[2], Harry Hamapumbu[2], Sungano Mharakurwa[1], Douglas E. Norris[1]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Macha Research Trust, Choma, Zambia

Malaria prevalence in Macha, southern Zambia has reduced significantly over the past decade, with community parasite prevalence currently below 1%. The province is now targeted for elimination strategies. It is important, however, to establish the potential of the local mosquito population to transmit malaria to estimate the risk of resurgence if the parasite were re-introduced. To address this, light trap collections of mosquitoes were carried out monthly in randomly selected geo-referenced households in the catchment area of Macha Hospital from February 2012 to February 2014. Peak times of host-seeking were studied using collection bottle rotators. Approximately 1200 female anophelines were collected from 856 traps across 336 different households. 60% were identified as the vector *An. arabiensis* to give a mean catch of 0.88 *An. arabiensis* per trap-night over the study period. Catches ranged from 0 to 148 *An. arabiensis* in a single trap, with highest catch recorded in February 2013 (monthly mean 7.2 per trap-night). Households with anophelines appeared to be clustered. Spatial analyses are ongoing to overlay mosquito distribution on malaria risk maps. 10.8% of *An. arabiensis* were blood fed and the human blood index was calculated as 0.93. At least 9 other anopheline species were identified, some of which were highly anthropophagic. One specimen was found to be positive for *Plasmodium falciparum* sporozoites by ELISA. Preliminary analysis of host-seeking times indicated peaks between 22:30 and 02:30, but vector activity was recorded as early as 20:30. Despite substantial reduction in malaria cases in Macha, large numbers of the vector *An. arabiensis* exist at certain times of the year and in certain localities. Here *An. arabiensis* demonstrates high endophily and anthropophagy. There is the potential for human exposure outside the times of net use. Whilst drug-based elimination strategies are encouraged, vector control methods should be maintained and entomological surveillance continued to monitor any increase in dominant or secondary vectors.

1630

OPTIMAL COVERAGE AT MINIMAL COST: A DYNAMIC MODELING APPROACH TO SIMULTANEOUS ALLOCATION OF MULTIPLE ANTI-MALARIA INTERVENTIONS

Beatrix Balogh[1], David W. Hutton[2], Ravi M. Anupindi[3], Leslie Arney[1], Meiting Liu[4], Haipeng Wu[4], Peter S. Larson[1], Prashant Yadav[1], Mark L. Wilson[4]

[1]William Davidson Institute, University of Michigan, Ann Arbor, MI, United States, [2]Department of Health Management and Policy, School of Public Health, University of Michigan, Ann Arbor, MI, United States, [3]Stephen M. Ross School of Business, University of Michigan, Ann Arbor, MI, United States, [4]Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, MI, United States

Malaria control efforts often involve a multipronged approach, including insecticide-treated nets (ITNs), indoor residual spraying (IRS), and pharmaceutical-based treatment and prevention. Public health agencies (government, private, NGOs) are constrained by conflicting demands on limited budgets. Determining the appropriate combination and magnitude of interventions is both complicated and context specific. Using a dynamic modeling approach, we analyzed the system dynamics of multiple malaria interventions functioning in concert, evaluated the cost-effectiveness of treatment and prevention strategies, and developed a resource allocation model to apportion resources across intervention types to maximize overall cost effectiveness. This compartmental mathematical model (using MATLAB) includes age, human immunity, and seasonality in the *Plasmodium* transmission cycle, with interventions of IRS, ITNs, intermittent preventive treatment for pregnant women (IPTp), and mass screening and treatment (MSAT). We assessed individual and combined effects on various transmission parameters such as biting rate, force of infection, and mosquito death. For a set of parameters considered typical of high transmission malaria endemic settings, results show that after five years into the model run and compared to a baseline with no interventions, with a combination of 65% coverage of IRS and ITNs, infection prevalence in children under five years of age and pregnant women would decrease by about 18%. Those above five years of age exhibited decrease in prevalence by about 16% with 70% combined IRS and ITNs. The addition of IPTp and MSAT decreased long-term community prevalence in all age groups by an overall additional 12%. This modeling approach allows a careful assessment and optimization of costs and benefits to particular combinations of malaria interventions and should assist public health groups in maximizing benefit under constrained budgets.

1631

HOUSEHOLD EXPENDITURES FOR MALARIA TREATMENT FROM A POPULATION-BASED SURVEY

Donald S. Shepard[1], Peter Mangesho[2], Robert Mpagala Kihomo[1], Aggrey Kihombo[1], Joseph Mugasa[2], Amos Malongo[2], Ruth Mnzava[2], Filemoni Tenu[2], Angelique K. Rwiyereka[1], Yara A. Halasa[1], Aaron M. Samuels[3], Mark Rowland[4], Frank Mosha[5], William Kisinza[2]

[1]Brandeis University, Waltham, MA, United States, [2]National Institute for Medical Research, Tanga, United Republic of Tanzania, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States, [4]London School of Hygiene & Tropical Medicine, London, United Kingdom, [5]Kilimanjaro Christian Medical Centre, Moshi, United Republic of Tanzania

Effective malaria control technologies not only improve health, but also reduce health expenditures by government and households. In Muheza, Tanzania, a randomized trial is assessing the effectiveness and cost-effectiveness of a non-pyrethroid insecticide-treated wall liner (ITWL) and indoor residual spraying (IRS). To project savings, the investigators are determining current expenditures for malaria treatment in conjunction with a cross-sectional epidemiological household survey. Following mapping and household enumeration, from December 2013 through February 2014 epidemiological teams visited 4200 randomly chosen

residents aged>6 months across 60 village clusters. Respondents who reported a malarial episode within the past 30 days were selected for a follow-up economic interview. Its questions included household income, treatment received, time required for travel and treatment, and expenses incurred. Overall, 18% of sampled residents reported a malaria illness episode within the last 30 days. Preliminary data are currently available for 467 representative malarial cases. Of these, 6% received inpatient hospital treatment, 91% received ambulatory treatment outside the home, and 3% received only in-home or no treatment. Overall combined travel and treatment time averaged 5 hours. Household expenditures per case averaged US$5.24 (TZS 8,545), but the median value of US$2.33 (TZS 3,800) was below the mean. This arose because expenditures on malaria treatment were highly skewed with a standard deviation equal to 213% of the mean. Analysis of household expenditures found 54% was for direct medical expense (consultations, beds, tests, and medications), 27% for transportation, and 19% for other expenses. Average expenditures of hospitalized patients US$32.82 (TZS53,491) were an order of magnitude above those of non-hospitalized patients of US$2.15 (TZS5,787). By comparison, household monthly revenue was under US$46.01 (TZS75,000) for 71% of respondents. Average inpatient expenses represent about a month's median income. Given low rural incomes, even routine medical expenses can strain household budgets. Adjustment of censored expenditures for episodes in progress and valuing time lost would increase estimated costs. If ITWL and IRS prove efficacious in this district, households will save on treatment expenses.

1632

IMPACT OF MALARIA INTERVENTIONS ON REDUCTIONS IN NEONATAL MORTALITY IN MALAWI, RWANDA AND MAINLAND TANZANIA

Rebecca Winter[1], Lia Florey[1], Anne Langston[2], Tom Pullum[1]

[1]The Demographic and Health Surveys Program, ICF International, Rockville, MD, United States, [2]ICF International, Rockville, MD, United States

Between 2000 and 2010 sub-Saharan Africa's impressive gains in under-five mortality have been accompanied by a more modest reduction in neonatal mortality. As a result, neonatal mortality now accounts for about a third of all under-five deaths in the region. This study selected three countries in SSA with significant reductions in NMR during this period_ Malawi, Rwanda, and mainland Tanzania_ in order to identify factors that contributed to the observed reductions, with special interest in the relative importance of scale-up of interventions to protect women against malaria during pregnancy. In Malawi, the neonatal mortality rate among women's most recent children born fell from 26 deaths per 1,000 live births in the five years preceding the 2000 DHS to 20 deaths per 1,000 live births in the five years preceding the 2010 DHS. In Rwanda, the NMR declined from 29 to 14 deaths per 1,000 live births, and in mainland Tanzania from 32 to 18 deaths per 1,000 live births between the 1999 and 2010 surveys. Multivariate decomposition procedures were used to examine the extent to which the scale-up of malaria interventions contributed to these observed reductions. Results show that in all three countries the rapid increase in mosquito net ownership was associated with the observed reductions in neonatal mortality, even after adjusting for changes in the distribution and effects of sociodemographic characteristics and key maternal and delivery services. In Malawi - where information on mothers' use of IPTp was available - the study did not find evidence that the scale-up of IPTp was associated with the reduction in NMR. In conclusion, the findings reinforce the importance of consistent and universal mosquito net use in areas with high prevalence of malaria. While malaria interventions are most often geared towards saving the lives of children at older ages (6 months to 5 years), the study findings contribute to a growing body of evidence pointing to the importance of malaria interventions to neonatal survival.

1633

FIGHT AGAINST MALARIA BY STUDENTS AND SCHOOLCHILDREN IN KINSHASA, DR CONGO

Sophia M. Mangala, Junior Musenga, Ornella L. Mampuya, Viviane Mitshiabu, Steve Tulantched, Thierry L. Bobanga, Thierry L. Bobanga

Protestant University of Congo, Kinshasa, Democratic Republic of the Congo

Malaria is a real public health problem in Africa. Despite the efforts made by countries, 655,000 deaths were recorded worldwide in 2010. And the Democratic Republic of the Congo accounts for nearly 10 % of the mortality And the fight against this disease should be of interest all groups in the community. And new and innovative approaches should be considered to reduce disease Thus, students of the Protestant University of Congo have been trained in the fight against malaria with the aim of training schoolchildren Students' knowledge was initially evaluated. And training was organized to provide knowledge about the transmission, prevention, particularly on ITN and finally what to do in case of fever. Method: 823 students in 9 schools in the city of Kinshasa sensitized by 20 university students who were trained by the managers of the National Program of Academic Medicine and University of Kinshasa Parasitology Department. The age of children was between 9 to 11 years old This awareness was marked by a strong interaction between children and sensitizers. At the end of awareness, the children were evalued to assess their understanding of the subject. And the best schoolchildren were awarded. It was noted that over 70 % of children could answer basic questions on malaria. In conclusion, at the end of this work, over 800 schoolchildren in the city of Kinshasa and 20 students have contributed to the fight against malaria, which is a tiny proportion of the population of said city. It is therefore important to continue these actions as awareness through children, future actor and relay in the transmission of knowledge from their family. This, could be, an innovative approach to malaria control.

1634

INSECTICIDE-TREATED NET (ITN) UTILIZATION AND MAINTENANCE IN KINSHASA, DR CONGO

Nono B. Mangaza[1], **Fabrice B. Ekomisa**[2], Solange E. Umesumbu[3], Billy S. Kunyu[4], Thierry L. Bobanga[2]

[1]University of Technology Belcampus, Kinshasa, Democratic Republic of the Congo, [2]Protestant University of Congo, Kinshasa, Democratic Republic of the Congo, [3]National Malaria Control Program, Kinshasa, Democratic Republic of the Congo, [4]National Institute of Biomedical Research, Kinshasa, Democratic Republic of the Congo

Malaria remains at the beginning of the first century killer in DRC with more than 60,000 deaths, especially among children under 5 and pregnant women Among the strategies, the LLIN is a tool of choice but its availability and hanging are operational problems. Furthermore its efficiency and sustainability are elements to evaluate. The present study was conducted with the aim to determine the proportion of households with at least one LLIN ; the proportion screens installed (used); the proportion of pregnant women who slept under LLINs ; the proportion of children who slept under LLINs ; determine the average age of the net and the number of washing on average per month. A cross-sectional study on the use and sustainability of ITN Long (LLINs) in the city of Kinshasa. We formed a cluster sampling and multistage. The sample size was 104 households per municipality. And 24 selected communes we got 2,512 households. The use of the net in Kinshasa was 59.4 %. Pregnant women and children under 5 were using respectively 70% and 60%. Through the city's most important use is in the center where the mosquito nuisance with the *Culex* is the greatest with 72.7 %. Whereas the periphery of the city use is low (44 to 55%) where anopheles populations are most abundant. The proportion of pregnant women and children under 5 years under nets was 70 % and 60 % respectively. In 2,512 households visited

nearly 4,812 LLINs were counted. The average duration of the net in the household was in the range 19 to 24 months with 1.5 washes by month. More than 50% of household use detergent for washing ITN. The majority of nets found in the households have probably lost their effectiveness before 18 months of utilization. In conclusion, the use of LLINs was still low in Kinshasa. The inhabitants do not respect manufacturer's recommendations in term of washing. Study on bio efficacy and durability in field use condition must be conducted to make evidence of efficacy

1635

EFFECT OF MASS DRUG ADMINISTRATION OF IVERMECTIN TO HUMANS ON MALARIA TRANSMISSION AND EPIDEMIOLOGY IN WEST AFRICA

Haoues Alout

Colorado State University, Fort Collins, CO, United States

Mass drug administration (MDA) of ivermectin to control filariasis and onchocerciasis has been shown to reduce malaria vector survivorship and the proportion of mosquitoes that are able to transmit *Plasmodium falciparum* in the same communities. Therefore, we have proposed that ivermectin MDA be considered in malaria control and elimination strategies. In our ongoing efforts to fully characterize the effect of ivermectin MDA on malaria transmission, we compared its effect on *Anopheles gambiae* and *Plasmodium falciparum* natural populations in three different West African countries (Senegal, Liberia and Burkina Faso) across different seasons. Blood fed mosquitoes were collected indoors before and after MDA in treated villages by health authorities for either lymphatic filariasis (ivermectin+albendazole) or for onchocerciasis control (ivermectin alone), and concomitant mosquito sampling was performed in untreated control villages. We compared the blood fed mosquito survivorship, sporozoite rate and parasite genetic diversity in mosquitoes, taking account for temperature, humidity, mosquito species and type of treatment. The mosquitocidal effect was consistent in all field sites and seasons, and did not vary with the addition of albendazole. The reduction in sporozoite rates were significant when compared to control villages but the observed reductions vary across field sites. In Burkina Faso in the treated village, sporozoite rate was significantly reduced by 79% following MDA (from 8% to 1.7%) and increased to pre-treatment levels after two weeks. Additionally, MDA completely eliminated sporozoite transmission from outdoor host-seeking mosquitoes for a period of two weeks. The potential impact of ivermectin on *Plasmodium* genetic diversity in mosquitoes and its consequence on malaria epidemiology and transmission will be discussed.

1636

IMPROVED ENVIRONMENTAL COMPLIANCE AND OPERATIONAL EFFICIENCY USING MOBILE SOAK PITS

Peter J. Chandonait

Abt Associates, Inc., Cambridge, MA, United States

The President's Malaria Initiative (PMI) employs thousands of spray operators and annually sprays millions of homes in Africa with a residual pesticide to kill malaria-bearing mosquitos. At the end of the work day, the spray operators must clean their spray tank and their personal protective equipment (helmets, face shields, gloves, and boots), resulting in wash water that is contaminated with pesticide residue. This contaminated water is treated before release in soak pits, which are pits 1 meter wide x 2 meters length x 1 meter deep, filled with a bed of commonly available cooking charcoal and other materials to filter out and break down the pesticide. These soak pits are centrally located within targeted spray areas so that many operators can travel to and use these facilities for clean-up at the end of the day. PMI has built and/or refurbished hundreds of soak pits over the past several years of IRS project implementation. Ensuring that these soak pits are built properly so as to minimize negative environmental impacts from IRS is a major environmental compliance responsibility of the implementing partner. However, in sparsely populated spray areas, teams may travel hours to reach targeted communities, and they may not be able to return to a centralized location for clean-up at the end of the day. AIRS has developed a mobile soak pit (MSP) that can be transported from site to site with the spray team, can be installed in less than one half hour, and can remove pesticide contamination from wash waters. Advantages of this mobile soak pit include a marked reduction in the time that spray operators spend traveling from site to wash area, better adsorption of the pesticide due to the characteristics of the filter material, better control of soak pit materials, and better protection of the community because the pesticide contamination is taken away in the filter.

1637

BASELINE STUDIES ON ANGLOGOLD ASHANTIS' INDOOR RESIDUAL SPRAYING PROGRAM (IRS) FOR MALARIA CONTROL IN GHANA

Kwaku P. Asante[1], Dominic B. Dery[1], David K. Dosoo[1], Kinsley Kayan[1], Sylvester Segbaya[2], Victor Asoala[3], Kofi Tchum[1], Frank Amoyaw[2], Nicholas Amoako[1], Abraham Oduro[4], Seth Owusu-Agyei[1]

[1]Kintampo Health Research Centre, Brong Ahafo Region, Ghana, [2]AngloGold Ashanti (Ghana) Malaria Control Limited, Ashanti Region, Ghana, [3]Navrong Health Research Centre, Upper East Region, Ghana, [4]Navrongo Health Research Centre, Upper East Region, Ghana

Malaria is still high in many parts of Africa including Ghana. Private -public partnership are essential in significantly reducing the burden of malaria in high risk countries. In Ghana, there is rapid scale up of indoor residual spraying in various parts of the country with funding from the Global Fund and the United States President's Malaria Initiative (PMI). AngloGold Ashanti (a mining company) is implementing an IRS programme in 25 districts in Ghana. The results of baseline studies (sensitivity of insecticides, prevalence of childhood malaria parasitemia and anemia) conducted prior to the IRS programme are presented here. Malathion was most effective with 100% *An. gambiae* mortalities in seven districts. Fenitrothion was effective in three districts whiles Propoxur worked in one district. Few (14) kdr susceptible strains were detected in samples analyzed with majority being homozygous kdrRR(120) resistant species compared to 32 Heterozygous kdrRS. Preliminary data shows high prevalence of malaria parasitemia (range: 30 - 50%) and anemia (range 40% - 60%). An organophosphate class of insecticide is considered most appropriate for IRS in eleven districts currently earmarked in Ghana. Rotation of different classes of insecticides over time is however recommended as it offers a practical solution for resistance management in light of rapid resistance development. Monitoring of malaria parasitemia and anemia during the IRS programme is required.

1638

TWO VARIANTS OF MULTIDRUG RESISTANT *VIBRIO CHOLERAE* O1 BIOTYPE EL TOR INVOLVED IN TWO CONSECUTIVES OUTBREAKS OF CHOLERA IN CAMEROON (2004 - 2005 AND 2010 - 2012)

Ariane A. Nzouankeu[1], Antoinette Ngandjio[1], Vanessa Nkamga[1], Pierrette Simo[1], Marie-Laure Quillici[2], Marie-Christine Fonkoua[1]

[1]Centre pasteur du Cameroun, Yaounde, Cameroon, [2]Institut Pasteur de Paris, Paris, France

Outbreaks of cholera due to toxigenic *Vibrio cholerae* are always dangerous and need a quick taking care of patient. From may 2010 to February 2012, an outbreak of cholera raged in Cameroon. The epidemic started in the North and reached the south of Cameroon 4 months later, in September 2010. The isolated strains, *V. cholerae* O1 serotype Ogawa, were multidrug resistant with additional resistance to nalidixic acid. isolated from 2 subsequent outbreaks of cholera in Cameroon, 2004 - 2005 and 2010 - 2011. Geographically, the 2004-2005 outbreak was localized in the south of Cameroon, while the current outbreak (2010-2011) covered the whole area of Cameroon. A total of 200 V. Cholerae O1

isolated during the last outbreak of cholera in Cameroon were used in this study; the strains belonged to biotype El Tor, serotype Ogawa. The strains were resistant to multiple antimicrobials especially nalidixic acid, which was the newest character. Molecular detection of their virulence factors revealed that tcpa gene which encodes the toxin coregulated pilus was characteristic to El Tor biotype, while nuleotide sequence of ctxB which encode the sub-unit B of the cholera toxin, was closer to the classical biotype. A total of 3 mutations was observed on the ctxB nucleotide sequence of which 2 were PFGE fingerprinting types showed different patterns, This study reveals that *V. cholerae* strains isolated in 2010-2011 were different from strains isolated in 2004-2005, using antimicrobial susceptibility phenotypes, characterization of antimicrobial resistance, cholera toxin genotyping, PFGE. PFGE analysis revealed two different unrelated profiles All the strains harboured tcpa El Tor allele, which was a supplemental argument ranging these strains in El Tor biotype.

1639

PREVALENCE OF ENTEROAGGREGATIVE *ESCHERICHIA COLI* VIRULENCE GENES IN YOUNG CHILDREN FROM RURAL SOUTH AFRICA AND PATHOGENESIS OF POTENTIAL VIRULENCE MARKERS IN A MOUSE MODEL: THE MAL-ED COHORT

Nicoline Tanih[1], David T. Bolick[2], Amidou Samie[1], Emanuel Nyathi[1], Leah Barrett[2], Relana Pinkerton[2], James P. Nataro[2], Araceli Santiago[2], Richard L. Guerrant[2], **Pascal O. Bessong**[1]

[1]University of Venda, Thohoyandou, South Africa, [2]University of Virginia, Charlottesville, VA, United States

Enteroaggregative *Escherichia coli* (EAEC) is recognized as a cause of growth shortfalls with or without diarrhea worldwide. A number of EAEC virulence-related genes (VRGs) have been described but their role in the clinical outcome of infection is not completely defined and may vary from one geographical location to another. Recently, we have characterized Aar (AggR activated regulator), a new negative regulator in EAEC. Deletion of Aar upregulated the expression of the EAEC master regulator AggR. Accordingly, we have found that an EAEC strain lacking Aar causes growth failure without diarrhea in our zinc deficient mouse model. We extracted DNA from 207 strains of EAEC isolated from the stool of 109 children followed from birth to 12 months of age from the Dzimuali community in the Limpopo Province of South Africa. We investigated the prevalence of EAEC VRGs using multiplex polymerase chain reaction. Samples were analyzed for identification of 18 VRGs. Plasmid encoded haemolysin (aar) was the most frequently detected (86.5%), followed by aggregative adherence regulator (*aggR*, 53.6%) and EAEC HilA homologue (*eilA*, 47.3%). Secreted autotransporter toxin gene (*sat*) was observed at lowest frequency (0%). Although only (5%) of participants had diarrhea in their first 12 months, children with EAEC had greater growth shortfalls (P= 0.03) and one child had an aar(-) EAEC with striking growth failure. These data confirm a high prevalence, endemicity and heterogeneity of EAEC strains in the Limpopo Province of South Africa and their association with growth failure. However, investigations are on-going to determine the impact of potential virulence determinants of the EAEC strains (aar (-) and *aggR* +) in a murine model and their impact on the host on diarrhea and growth impairment.

1640

SENDING PEACEKEEPING TROOPS INTO AN ONGOING CHOLERA OUTBREAK - THE CHILEAN EXPERIENCE DURING MINUSTAH (MISSION DES NATIONS UNIES POUR LA STABILISATION EN HAÏTI)

Reinaldo Rosas[1], Alberto Fica[1], Dannette Guiñez[1], Myriam Polanco[2], Alejandra Saldivia[1], Wilson Wigant[1], Vjera Triantafilo[1], Thomas Weitzel[3]

[1]Hospital Militar de Santiago, Santiago, Chile, [2]Comando de Salud del Ejército, Santiago, Chile, [3]Universidad del Desarrollo Clinica Alemana, Santiago, Chile

Since 2004, Chile participates in MINUSTAH with a permanent contingent of 500 soldiers, who change every 6 months. The Preventive Medicine Service prepares troops and medical units for potential threats. After the 2010 earthquake, a cholera outbreak rapidly spread through Haiti, which was probably related to soldiers participating in the same UN mission. This work describes the experience with pre- and post-deployment measures of the Chilean Forces to reduce the cholera risk in Haiti as well as importation into Chile by asymptomatic carriers. Those actions included sanitary and hygiene education of troops, training for medical officers, pre-deployment immunizations with whole-cell oral cholera vaccine (WC/rBS), and post-deployment microbiological surveillance. In 2011, vaccine side effects were monitored using a standardized questionnaire. After deployment, all troops were screened for *Vibrio cholerae* carriership. Data on WC/rBS vaccination were available for 569 soldiers of whom 9.7% reported systemic and 10.5% gastrointestinal (GI) side effects. A shorter interdose interval (7 vs 30 days) was associated with more GI disturbances (17.4% vs 7.7%, $p<0.001$). In the subgroup receiving WC/rBS 30 days apart, systemic side effects were more common if peacekeepers simultaneously received other pre-deployment vaccines (11.9% vs 4.3%, $p<0.05$). All surveillance stool cultures were negative, except for 2 (in 2012) which both grew strains of non-pathogenic *V. cholerae.* Cholera is still a threat for underdeveloped countries and military operations within these countries need to take measures to prevent infection and further spread the disease by deployed troops. Our experience showed an acceptable safety profile of WC/rBS, especially if doses are separated by 30 days. This vaccine provides a fairly high protection rate for the first 6 months, but its influence on the rate of asymptomatic carriers is uncertain. Therefore, additional post-deployment stool cultures seem an appropriate surveillance tool for returnees to non-industrialized countries such as Chile.

1641

NOVEL IMMUNOLOGICAL SIGNALS FOR DIAGNOSING ACUTE TYPHOID FEVER

Nga Tran Vu Thieu[1], Elizabeth Klemm[2], Gordon Dougan[2], Sabina Dongol[3], Buddha Basnyat[3], Stephen Baker[1]

[1]Oxford University Clinical Research Unit, Vietnam, Ho Chi Minh, Vietnam, [2]Sanger Institute, Cambridge, United Kingdom, [3]Oxford University Clinical Research Unit, Nepal, Kathmandu, Nepal

Salmonella Typhi is the causative agent of typhoid. There are a variety of methods for diagnosing typhoid; all lack sensitivity and specificity. Serological assays targeting LPS and/or flagella are commonly used settings to diagnose typhoid. However, cross-reactivity makes these antigens unsuitable for assessing disease burden and diagnosing acute illness. There have been few studies focusing on studying the immunological response to specific *Salmonella* antigens. Using an *S.*Typhi antigen array we identified a number of antigens that elicited a significant IgM or IgG response greater than that of uninfected controls. We selected the best twelve (6 IgM and 6 IgG) antigens that gave a differential response between the groups for further investigation. Firstly, we expressed and purified these antigens and immunized mice to study the ability of the mouse serum to stimulate bacterial killing. All of the serum samples from immunized mice were able to stimulate a bactericidal response against *S.* Typhi, inhibiting >80% of the bacterial growth over three hours.

Additionally, eight of the serum samples had a bactericidal effect on *S.* Paratyphi A. None of the serum from immunized mice demonstrated any bactericidal activity against *S.* Typhimurium. We further investigated this bactericidal response by repeating the experiments with gene knockout strains. There was a marked reduction in bactericidal activity with the immunized mouse serum on the strains of *S.*Typhi strains harboring the respective specific antigen encoding gene knockout. Specifically, serum from mice immunized with CdtB (subunit of typhoid toxin) demonstrated a significant reduction bactericidal activity against a *cdtB S.*Typhi mutant in comparison to wild-type *S.*Typhi. These preliminary data show that *S.*Typhi 12 antigens appear to stimulate specific and strong immunological responses in patients with acute typhoid. Furthermore, we suggest these antigens may be candidate diagnostics or subunit vaccines, and provide a novel insight for further understanding of host immune responses induced during acute typhoid.

1642

THE SPATIOTEMPORAL DYNAMICS AND PHYLOGENETICS OF *SALMONELLA PARATYPHI* A IN KATHMANDU, NEPAL

Duy Pham, Stephen Baker

Oxford University Clinical Research Unit, Ho Chi Minh, Vietnam

Enteric fever is a life threatening systemic disease caused by the bacteria *Salmonella* Typhi and *Salmonella* Paratyphi A, B, and C. Typhi is the major agent of enteric fever, yet Paratyphi A is emerging at an unprecedented rate. In our location in Patan Hospital, Kathmandu, standardised blood culture surveillance over the last ten years has shown annual increases in the proportions of individuals with Paratyphi A. More than two thirds of the culture confirmed enteric fever cases are now caused by this serovar. Both Typhi and Paratyphi A are systemic pathogens that induce indistinguishable syndromes. However, they exhibit contrary epidemiologies, different geographical distributions, and different propensities to develop resistance to antimicrobials. Additionally, they are genetically and phenotypically distinct, having gone through a lengthy process of convergent evolution to cause an identical disease. To understand the emergence and the molecular epidemiology of Paratyphi A in our setting we genome sequenced 182 organisms isolated from patients with acute or relapsed enteric fever, and a number isolated from the gallbladder of asymptomatic carriers. Performing phylogenetic analysis and evolutionary reconstruction we find that Paratyphi A is isolated and genetically distinct from Typhi. Our data show that Paratyphi A has been through a major clonal expansion in Kathmandu, apparently driven by resistance to fluoroquinlones and increased virulence through multi-copy effector proteins. Contemporary isolates of Paratyphi A have been introduced from other parts of Asia and induced a clonal replacement of the native strain(s). We surmise that Typhi and Paratyphi A have a dissimilar epidemiology in Nepal with Paratyphi A associated with spatiotemporal outbreaks and person-to-person transmission. Our study is the first to tackle the local phylogenetics and spatiotempral dynamics of Paratyphi A. Our work outlines new perspectives on enteric fever and will pave the way for future genomic epidemiology investigations of this important emergent pathogen.

1643

INCIDENCE AND ETIOLOGY OF INFECTIOUS DIARRHEA FROM A MULTIYEAR FACILITY-BASED SURVEILLANCE SYSTEM IN GUATEMALA, 2008-2012

Wences Arvelo[1], Aron Hall[1], Olga Henao[1], Beatriz Lopez[2], Chris Bernart[2], Juan C. Moir[3], Lissette Reyes[3], Susan P. Montgomery[1], Oliver Morgan[1], Alejandra Estevez[2], Maria R. Lopez[2], Michele B. Parsons[1], Jan Vinje[1], Nicole Gregoricus[1], Jon Gentsch[1], Umesh Parashar[1], Eric Mintz[1], Joe Bryan[1], Kim A. Lindblade[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Universidad del Valle de Guatemala, Guatemala City, Guatemala, [3]Ministry of Health and Welfare, Guatemala City, Guatemala

Diarrheal diseases are a major cause of morbidity and mortality worldwide, yet data on etiology and population-level incidence in developing countries are limited. Diarrhea surveillance was conducted at two hospitals and 10 ambulatory clinics in the departments of Santa Rosa and Quetzaltenango in Guatemala. A case was defined as a person of any age having ≥3 loose stools in a 24-hour period and was admitted or presented to the surveillance facilities. Epidemiologic and clinical data were collected. Stool specimens were tested for bacterial, parasitic, and viral enteric pathogens. Estimated incidence rates were calculated by adjusting for healthcare seeking behaviors, based on results from a survey in the surveillance area assessing the proportion of those with reported diarrhea who visited a surveillance facility during their illness. From November 2008 to December 2012 there were 5,331 diarrhea cases. The weighted estimated community incidence averaged 659 diarrhea cases per 10,000 persons per year during the four year period. The estimated incidence was highest among children aged <5 years, averaging 1,584 cases per 10,000 children per year, while among those aged ≥5 years the estimated incidence averaged 311 cases per 10,000 persons. From 2008-2009 samples from 1,401 (26%) cases were tested for all the pathogens of interest. Among these, 846 (60%) specimens were from children aged <5 years in whom a virus was identified in 211 (25%) patients; of which, 178 (84%) tested positive for norovirus and 101 (48%) for rotavirus, including co-infections. Among the 555 patients aged ≥5 years the most frequently identified etiology was bacterial with 134 (24%) cases. Diarrheagenic *Escherichia coli* was detected in 94 (70%) cases, *Shigella* spp in 31 (23%), *Campylobacter* spp in 5 (4%), and *Salmonella* spp in 4 (3%) cases. Identification of parasites was low (24 cases, 9%), and most cases were among those aged 5-19 years. These data demonstrate a substantial burden of viral and bacterial diarrheal diseases in Guatemala, which may help guide public health policies aimed at reducing the burden of illness and death due to diarrhea.

1644

SALMONELLA ENTERICA SEROVAR ENTERITIDIS OUTBREAK AT A LODGE IN MOKOPANE, LIMPOPO PROVINCE, JANUARY 2014

Akhona N. Tshangela

National Institute for Communicable Diseases, Johannesburg, South Africa

Salmonella enterica serovar Enteritidis is a leading cause of foodborne disease worldwide, but there is little data available in South Africa. Asymptomatic food-handlers have also been associated as a source of infection. We investigated the aetiology of an acute gastroenteritis outbreak among persons staying at a lodge in Limpopo Province, South Africa in January 2014. A retrospective cohort analysis was used to determine the risks of illness associated with consuming foods and/or beverages at the lodge. The at-risk population were contacted to complete a standard questionnaire related to food and beverages consumed at the lodge, symptoms of illness, visits to healthcare facilities and specimen submission for pathogen testing. Food and water samples were tested, as well as completion of an environmental assessment questionnaire by staff and external caterers. The data was categorised and STATA version 12 was used for multivariate analyses. A total of 73 ill persons, including

3 laboratory-confirmed infections, were identified: 69/109 (63%) of the selected cohort were seen at health facilities. Of the at-risk population 87% (109/124) completed the standard questionnaire: 66 cases of gastrointestinal illness and 43 healthy individuals were identified, with a corresponding attack rate of 61%. Most of the cases were females (86%, n=57) with a mean age of 33 years (S.D=7.1), and 36% (n=24) of the cases were hospitalised. Epidemiological data suggested a point source outbreak with no further transmission. Statistical analysis of survey data indicated consumption of diluted fruit juice (from concentrate) adjusted by other food and beverage items, presented a risk ratio of 1.5 (95% CI, 1.1-1.8, p=0.032). Environmental analysis indicated increased risks for cross-contamination. The outbreak was possibly due to cross-contamination of food/ beverages prepared in the lodge kitchen, and fruit juice consumption was the main exposure associated with ill cases. Feedback on food safety and hygiene practices to prevent cross-contamination at the kitchen lodge were provided.

1645

FECAL SHEDDING OF ROTAVIRUS FOLLOWING ROTARIX VACCINATION IN A COHORT OF BOLIVIAN INFANTS

Rachel M. Burke[1], Paulina A. Rebolledo[2], Anna Fabiszewski de Aceituno[1], Lucia Inchauste[3], Volga Iñiguez[3], Rita Revollo[4], Parminder Suchdev[1], Juan Leon[1]

[1]Rollins School of Public Health, Emory University, Atlanta, GA, United States, [2]Emory University School of Medicine, Atlanta, GA, United States, [3]Universidad Mayor de San Andrés, La Paz, Plurinational State of Bolivia, [4]Hospital Municipal Los Andes, El Alto, Plurinational State of Bolivia

Worldwide children under the age of five suffer from an estimated 138 million cases of rotavirus (RV) diarrhea per year. Currently available oral RV vaccines, such as the human attenuated monovalent vaccine Rotarix, increase RV-specific immunity and prevent severe diarrhea. Following vaccination, RV vaccine strains are expected to replicate in the intestinal tract and be excreted in feces. Fecal shedding of vaccine strains could lead to horizontal transmission of such strains and theoretically afford protection to unvaccinated contacts, which would be of substantial benefit in impoverished countries such as Bolivia where rates of immunization are suboptimal or RV associated morbidity is high. With the ongoing rollout of RV vaccines in low-middle income countries, it is imperative to evaluate and describe RV vaccine-virus shedding in vaccine recipients. Between June 2013 and April 2014 a birth cohort of 462 Bolivian infants were enrolled and followed through receipt of Rotarix to quantify the prevalence of fecal shedding within 7 days of the initial vaccine dose (at approximately 2 months of age). Shedding was assessed using enzyme immunoassay (EIA) and in a subset of infants real-time reverse transcription-polymerase chain reaction (RT-PCR) was used for confirmation. Bivariate logistic regression was used to identify potential predictors of shedding. The mean age at the initial (pre-vaccine) visit was 35 days (SD 8 days), and 55% of the infants were male. Baseline prevalence of stunting was 20%, prevalence of preterm birth was 19%, and prevalence of low birth weight was 7%. The mean maternal age was 26 years (SD 6 years), and 61% of mothers had completed secondary school. Shedding was identified in 6 infants out of 305 tested (2%). All before-mentioned predictors were tested, but no significant associations with shedding were detected (likely due to lack of power). Initial hypotheses for the low shedding rate include the high prevalence of exclusive breastfeeding, role of maternal antibodies, and circulating RV in the community; these and other potential explanations will be addressed in future investigation.

1646

INTESTINAL INFLAMMATION AND ALTERED BONE METABOLISM IN PERUVIAN INFANTS

Gwenyth Lee, Pablo Peñataro Yori, Margaret Kosek

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

Low-grade inflammation resulting from frequent enteric episodes and resulting enteropathy is believed to be a causes of growth faltering among children in the developing world. In order to clarify these associations, markers of bone metabolism may be beneficial, as they are more dynamic over short intervals than anthropometry. In order to test this hypothesis, urine samples from 139 6-month olds and 64 15-month old Peruvian Amazonian infants, and serum samples from the same children at 7 and 15 months, were tested for markers of bone collagen formation (plasma osteocalcin (OC)) and resorption (urinary deoxypyridinoline (DPD)/ creatinine (Cr)), as well as the acute phase protein plasma alpha-1-acid glycoprotein (AGP) and the plasma cytokines TNF-α, interleukin-6 (IL-6), and IL-1β , and fecal alpha-1-antitrypsin (AAT), a marker of intestinal inflammation. The mean plasma osteocalcin at seven months was 41.2µg/L in boys and 36.4µg/L in girls, by 15 month this had fallen to and 34.1 µg/L and 25.2 µg/L, respectively. The mean DPD/Cr at 6 months was 58.9 nmol/mmol Cr and 65.1 nmol/mmol Cr for boys and girls, respectively, and at 15 months, 68.3 nmol/mmol Cr and 52.1 nmol/mmol Cr for boys and girls, respectively. The mean length-for-age Z score (LAZ) at 6 months was -1.3 and 19.4% were stunted (LAZ< -2). By15 months of age the mean LAZ was -2.0 and 53.1% were stunted. The mean weight-for-length Z-score (WLZ) was 0.9 at 6 months , 0.8 at 7 months, and 0.4 at 15 months. 76.4% of children at 6mo, and 69.1% at 15 month were classified as having subclinical inflammation, defined by AGP> 1g/L. Bone collagen metabolism was altered by nutritional status, as OC was inversely associated with both length-for-age and weight-for-length, and DPD/Cr was positively associated with length-for-age. Correspondingly, the ratio of OC/DPD was highest among shorter and leaner children. After adjusting for anthropometric status, age, and gender, OC and the ratio of OC/DPD were both inversely associated with fecal AAT, but not with plasma AGP, tnf-alpha, IL-6, or IL-1beta. Our findings suggest that bone metabolism is suppressed among children with chronic intestinal inflammation.

1647

EPIDEMIOLOGY OF SELF-REPORTED HEALTH EVENTS AMONG DEPLOYED U.S. MILITARY PERSONNEL

Ruvani M. Chandrasekera[1], Mark S. Riddle[2], Chad K. Porter[2]

[1]Cherokee Nation Technology Solutions, Silver Spring, MD, United States, [2]Naval Medical Research Center, Silver Spring, MD, United States

Deployed military personnel are at risk of experiencing numerousadverse health events to include diarrhea, vomiting, fever and muscle aches while on humanitarian and war-fighting operations. These symptoms can negatively impact servicemember effectiveness and epidemiologic studies are needed to describe their frequency and associated risk factors; however, extensive prospective epidemiological studies in operational environments are challenging. We utilized self-reported data collected through the Post-Deployment Health Assessment from individuals following operational deployments and compared prevalence estimates across region/country of deployment, multiple demographic characteristics as well as pre-existing medical conditions. Univariate and multivariate logistic regression methods were also used to identify unique risk factors while controlling for important covariates. Of 21,982 subjects, the top five self-reported symptoms included back pain (15.9%), feeling tired/problems sleeping (15.7%), swollen, stiff or painful joints (13.6%), diarrhea (12.7%) and muscle aches (11.1%). Among those reporting diarrhea/vomiting, a high proportion were assigned to limited duty/bed rest (36% and 54%, respectively). Further data will be presented on the estimated level of care required for reported adverse health events, and potential risk factors for increased self-report of diarrhea and vomiting. While these results are limited to self-report, the data support prior studies highlighting diarrhea

and vomiting as significant causes of morbidity and troop down-time during operational deployment. Furthermore, recent studies highlighting the link between acute gastroenteritis and long-term adverse health outcomes raise the importance of these common, deployment-related health events. Continued evaluation of primary prevention strategies is needed.

1648

REGULATION OF SMALL RHO GTPASES IN REDUCING INTESTINAL CELLS MIGRATION INDUCED BY STRAINS OF ENTEROPATHOGENIC *ESCHERICHIA COLI*

Paloma A. Cavalcante[1], Mara M. Prata[1], Antônio V. Silva[1], Josiane S. Quetz[1], Alexandre B. Havt[1], Ila F. Lima[1], Eunice B. Carvalho[1], Pedro H. Quintela[1], Tamara S. Rodrigues[1], Rafael H. Gonzaléz[1], Rosemeyre S. Freire[1], Sean R. Moore[2], James Nataro[3], Richard L. Guerrant[3], **Aldo A. Lima**[1]

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

Intestinal epithelial migration provides an important early response when intestinal pathogens damage the host intestinal barrier. Small Rho GTPases Rac1, RhoA and Cdc42 are important regulators of this migratory response. We sought to test the hypothesis that EPEC impairs intestinal epithelial migration *in vitro* via small Rho GTPase-dependent mechanisms. Methods: We investigated the effects of EPEC strain E2348/69, EPEC strain LDI001 (isolated from a malnourished child), and commensal E. coli HS on IEC-6 cell migration; as well as on the regulation of transcription and gene expression of small Rho GTPases by qPCR and confocal immunofluorescent microscopy, respectively. Results: We observed a significant reduction in IEC-6 cell migration for all E. coli strains tested. However, pathogenic EPEC strains reduced migration to a greater degree than E. coli HS. Only EPEC E2348/69 induced significant cellular necrosis. Gene analyses of small Rho GTPases revealed an increase in rac1 transcription in EPEC LDI001 infected cells and upregulation of rhoA transcription following infection with all strains. Confocal imaging showed an increase in Rac1 expression and decrease in RhoA in response EPEC LDI001 infection. We further observed increased expression of Cdc42 in all infected groups. Conclusions: The results suggest differential suppression of migration and co-regulation of small Rho GTPases in response to infection with enteropathogenic vs. commensal E. coli strains. These *in vitro* data corroborate an emerging *in vivo* and clinical understanding of the pathobiology of this infection and its associations with malnutrition and intestinal barrier injury.

1649

INCORPORATING HERD PROTECTION INTO A COST-BENEFIT ANALYSIS OF TYPHOID FEVER VACCINE INTERVENTIONS

Marina Antillon, Virginia E. Pitzer

Yale University, New Haven, CT, United States

Recent studies have shed light on the costs and savings of typhoid fever vaccination campaigns, but no studies been able to quantify the potential economic savings attributable to realistic estimates of herd protection, the protection conferred to individuals who did not receive the vaccine. In light of numerous budget constraints in resource-poor settings, it is necessary to more accurately estimate the indirect effects of vaccine campaigns as well as the additional potential financing mechanisms that may lower the costs of vaccination per DALY saved. Field studies are now available to assess the burden of typhoid fever and the possible impact of Vi-polysaccharide and Vi-conjugate vaccines. Using mathematical models for typhoid transmission, we can quantify the indirect protection of vaccines under different vaccine strategies. Moreover, surveys on the private demand for these vaccines in South Asian contexts, where the disease is endemic, inform calculations on the optimal vaccine subsidies necessary to achieve a desired level of vaccine coverage while recuperating some of the programmatic costs through user fees. With that in mind, we will show that past estimates of the costs of vaccination per life year saved have been overestimated when compared to an analysis that takes into account accurate estimates of indirect vaccine protection at different pricing levels.

1650

CHARACTERIZING THE REGIONAL AND GLOBAL DISTRIBUTION AND BURDEN OF CHOLERA

Sean M. Moore, Andrew A. Azman, Carla E. Zelaya, Justin Lessler

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

There are an estimated 2.8 million cholera cases per year globally, but the majority of these cases are not detected or reported. Because of inadequate surveillance and reporting the global distribution of cholera risk and its public health burden are poorly known. Previous attempts to determine the global burden of cholera have relied on a limited number of case studies that do not capture the broad range of settings and environmental conditions where cholera occurs. Here we assemble a database of cholera surveillance and incidence reports from a variety of government, scientific, and non-governmental agency sources, with a particular focus on sub-Saharan Africa where a majority of cholera cases have been reported in the past several decades but where the distribution of risk and burden is still poorly understood. We will then use a formal modelling framework to associate cholera transmission with environmental and socioeconomic variables and map the global distribution of cholera risk and incidence. As a preliminary analysis we developed cholera incidence maps for the West African country of Guinea-Bissau using a hierarchical Bayesian framework with cholera data at spatial scales ranging from neighborhood-level incidence in the capital city of Bissau to country-level reports. Cholera incidence from 1986-2009 was highest in the island and coastal districts (including Bissau city). Incidence did not change significantly between the 1990s (which included outbreaks in 1994 and 1996-1997) and the 2000s (which included outbreaks in 2005 and 2008), except for an increase of 26-280% in the islands of the Bijagos Archipelago. Improving our understanding of the spatial distribution of cholera in Guinea-Bissau and associating incidence with climate, environmental and socioeconomic factors will provide a basis for planning public health preventions to reduce cholera transmission in this region.

1651

BIOMARKERS OF ENVIRONMENTAL ENTEROPATHY FOR POSITIVELY ASSOCIATED WITH TOXIN-SPECIFIC B AND T CELL RESPONSES TO AN ORAL CHOLERA VACCINE IN BANGLADESHI CHILDREN

Daniel T. Leung[1], Taufiqur R. Rahman[2], Muhammad Ikhtear Uddin[2], Naoshin S. Nishat[2], Mohammad Rubel Hoq[2], Amit Saha[2], Jason B. Harris[1], Stephen B. Calderwood[1], Firdausi Qadri[2], Edward T. Ryan[1]

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

Environmental enteropathy (EE) is a poorly understood condition that refers to chronic alterations in intestinal permeability, absorption, and inflammation that affects young children in resource limited settings. While EE has been linked to suboptimal oral vaccine performance in children, the causative immunological mechanisms are poorly defined. The objective of this study was to determine how markers of enteropathy are associated with immune responses to an oral cholera vaccine (OCV). We collected blood and stool from 40 Bangladeshi children who received two doses of an OCV given 14 days apart. We measured five EE markers, including stool myeloperoxide (MPO), a marker of intestinal inflammation, stool alpha anti-trypsin (AAT), a marker of intestinal absorption, as well as plasma endotoxin core antibody (EndoCab), plasma intestinal fatty acid binding protein (iFABP), and plasma soluble CD14 (sCD14), all markers of microbial translocation. We measured cholera toxin (CT)- and lipopolysaccharide

(LPS)-specific antibody responses by ELISA, toxin-specific memory T cell responses by flow cytometry following whole blood culture, and T cell culture cytokines by Luminex array. Using a multiple linear regression model, we assessed each vaccine-associated immune response outcome as separate dependent variables, and used log-transformed EE marker measurements, along with gender, blood group, and age, as independent variables. We found stool MPO to be a positive predictor of antibody responses to CT, plasma iFABP a positive predictor of gut-homing memory T cell responses, and stool AAT a positive predictor of interferon-gamma responses. No marker predicted antibody responses to LPS. Variance inflation factor for all independent variables were < 1.6, suggesting no multi-collinearity. In summary, we demonstrate that biomarkers of environmental enteropathy are positive predictors of toxin-specific immune responses to an OCV.

1652

LEPROSY IN NIGERIA (2008-2012): AN EVALUATION OF THE NATIONAL SURVEILLANCE SYSTEM

Aishatu Abubakar Sadiq[1], Joshua A. Obasanya[2], Patrick Nguku[1], Gabrielle Poggensse[1], Kabir Sabitu[3]

[1]Nigeria Field Epidemiology and Laboratory Training Programme, Abuja, Nigeria, [2]National Tuberculosis and Leprosy Control Programme, Abuja, Nigeria, [3]Department of Community Medicine, Ahmadu Bello University, Zaria, Nigeria

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* an acid fast, rod shaped bacillus. It was historically associated with social isolation and psychological consequences. Although eradicated in most countries, ongoing transmission in African and Asian countries requires enhanced surveillance and monitoring. In 2012 Nigeria reported 3805 cases and no deaths. We evaluated the national Leprosy surveillance system to assess its usefulness and attributes. Centers for Disease Control(CDC) guidelines for evaluating surveillance systems were used. Ten Stakeholders from partner agencies, national and state level were interviewed using structured questionnaires. Secondary data (2008-2012) was abstracted from surveillance data submitted to the National Tuberculosis and Leprosy Control Program. Laboratory diagnostic capacity was also assessed.Out of 20,623 cases reported, 3805(18.45%) were received in 2012, 3623(17.57%) in 2011,3913(18.97%) in 2010,4383(21.25%) in 2009 and 4899 (23.76%) in 2008.The prevalence reduced from 4.3x10-3 /10,000 population in 2008 to 1.2x10-4/10,000 population in 2011.Child proportion was 10.7% in 2008 and 8.0% in 2011.Grade 2 disability rate ranged between 11.7% and 13.4%.Being an adult (OR=1.97; 95% CI =1.37-2.82) and male (OR=1.25; 95% CI=1.05-1.48) was found associated with Multibacillary Leprosy. Residents of the Northwest region (OR=0.73; 95%CI=0.59-0.90) were less likely to have Multibacillary Leprosy. The system is active and rated simple by 17(85%) of respondents. Review of weekly,quarterly and monthly reporting forms and records at the national level showed timeliness. All suspected cases (100%) were laboratory tested within 24hours of presentation.There is a high laboratory turnover of staff and low numbers of personnel trained in laboratory diagnosis.The system is fully integrated with surveillance of Tuberculosis at all levels. Leprosy transmission is still ongoing and the WHO elimination target (<1 case/10,000 population) has been achieved at the national level. Pockets of leprosy exist in the northwest region.We recommend intensification of surveillance activities in all zones,improvement of Laboratory diagnostic capacity and recruitment of additional personnel. The system is acceptable,flexible, simple and timely. The system is meeting its purpose and promotes the achievement of the global elimination target for leprosy.

1653

OPTIMIZING THE CHEMOTHERAPEUTIC APPROACH FOR THE TREATMENT OF BURULI ULCER: POSSIBLE OPTIONS AND RESEARCH NEEDS

Charitha Gowda[1], Martina Casenghi[2], Julien Potet[2], Eric Comte[3], Jennifer Cohn[2]

[1]University of Pennsylvania, Philadelphia, PA, United States, [2]Medecins Sans Frontieres Access Campaign, Geneva, Switzerland, [3]Medecins Sans Frontieres, Geneva, Switzerland

Buruli ulcer (BU) is a serious necrotising skin infection caused by the environmental pathogen *Mycobacterium ulcerans*, and represents the third most common mycobacterial infection. Current WHO treatment has simplified the delivery of care for BU by recommending that early, limited lesions be treated with antibiotics alone. However, these recommendations still present significant disadvantages including the use of injectable agents. There is an urgent need to identify alternative oral regimens that are effective, short-course, have few drug-drug interactions with antiretrovirals and can be used in children. We performed a systematic literature review for publications focused on chemotherapy for *M. ulcerans* infection. We searched PubMed, EMBASE, Scopus, WHO Global Index Medicus, CAB Abstracts and Cochrane Library with standardized search terms, screened abstracts submitted to international conferences and assessed the ClinicalTrials.gov registry. While there were no restrictions by publication date or type, only articles in English, French and Italian as of December 31, 2012 were included. We included *in vitro* and clinical studies, with the primary outcomes of clinical resolution of the ulcer without surgery (clinical studies) and assessment of *in vitro* activity (pre-clinical data). We excluded all studies without microbiological confirmation of *M. ulcerans* infection. 49 clinical studies including 6 RCTs, 14 observational cohorts, 11 case series and 18 case reports were identified. Various drugs and drug combinations were identified as having clinical efficacy against BU disease in resource-poor settings. In particular, the combinations of clarithromycin+rifampin and clarithromycin+fluoroquinolones demonstrated good efficacy and safety. *In vitro* data reveal a number of promising compounds. Although recent studies indicate that a fully oral regimen for BU may be as equally effective as regimens containing aminoglycosides, further research is needed to identify and evaluate new treatments. The anti-tuberculosis research & development (R&D) pipeline represents a potentially rich source of novel compounds for BU treatment. We propose an R&D agenda aimed at delivering new, more efficacious and readily implementable treatments against Buruli ulcer in resource-limited settings.

1654

PERSISTENT GUT MICROBIOTA IMMATURITY IN MALNOURISHED BANGLADESHI CHILDREN

Sathish Subramanian[1], Sayeeda Huq[2], Tanya Yatsunenko[3], Rashidul Haque[2], Mustafa Mahfuz[2], Mohammed A. Alam[2], Amber Benezra[4], Joseph DeStefano[3], Martin F. Meier[3], Brian D. Muegge[3], Michael J. Barratt[3], Laura G. VanArendonk[3], Qunyuan Zhang[5], Michael A. Province[5], William A. Petri[6], Tahmeed Ahmed[2], Jeffrey I. Gordon[3]

[1]Center for Genome Sciences and Systems Biology, Washington University, St. Louis, MO, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [3]Center for Genome Sciences and Systems Biology, Washington University, St. Louis, MO, United States, [4]Department of Anthropology, New School for Social Research, New York, NY, United States, [5]Division of Statistical Genomics, Washington University, St. Louis, MO, United States, [6]Departments of Medicine, Microbiology and Pathology, University of Virginia School of Medicine, Charlottesville, VA, United States

Therapeutic food interventions have reduced mortality in children with severe acute malnutrition (SAM) but incomplete restoration of healthy growth remains a major problem. The relationships between the type of

nutritional intervention, the gut microbiota, and therapeutic responses are unclear. In the current study, bacterial species whose proportional representation define a healthy gut microbiota as it assembles during the first two postnatal years were identified by applying a machine-learning-based approach to 16S rRNA datasets generated from monthly fecal samples obtained from a birth-cohort of children, living in an urban slum of Dhaka, Bangladesh, who exhibited consistently healthy growth. These age-discriminatory bacterial species were incorporated into a model that computes a 'relative microbiota maturity index' and 'microbiota-for-age Z-score' that compare development (defined here as maturation) of a child's fecal microbiota relative to healthy children of similar chronologic age. The model was applied to twins and triplets (to test for associations of these indices with genetic and environmental factors including diarrhea), children with SAM enrolled in a randomized trial of two food interventions, and children with moderate acute malnutrition. Our results indicate that SAM is associated with significant relative microbiota immaturity that is only partially ameliorated following two widely used nutritional interventions. Immaturity is also evident in less severe forms of malnutrition and correlates with anthropometric measurements. Microbiota maturity indices provide a microbial measure of human postnatal development, a way of classifying malnourished states, and a parameter for judging therapeutic efficacy. More prolonged interventions with existing or new therapeutic foods and/or addition of gut microbes may be needed to achieve enduring repair of gut microbiota immaturity in childhood malnutrition and improve clinical outcomes.

1655

CONTRIBUTION OF THE COMMUNITY HEALTH VOLUNTEERS IN THE CONTROL OF BURULI ULCER IN BENIN

Yves T. Barogui[1], Ghislain E. Sopoh[2], Roch C. Johnson[3], Janine de Zeeuw[4], Ange D. Dossou[2], Jean G. Houezo[2], Annick Chauty[5], Julia Aguiar[6], Didier Agossadou[7], Patrick A. Edorh[3], Kingsley Asiedu[8], Tjip S. van der Werf[4], Ymkje Stienstra[4]

[1]Centre de Dépistage et de Traitement de l'Ulcère de Buruli de Lalo, Lalo, Benin, [2]Centre de Dépistage et de Traitement de l'Ulcère de Buruli d'Allada, Allada, Benin, [3]Université d'Abomey-Calavi, Abomey-Calavi, Benin, [4]University of Groningen, University Medical Center Groningen, Department of Internal Medicine/Infectious Diseases, Groningen, Netherlands, [5]Centre de Dépistage et de Traitement de l'Ulcère de Buruli de Pobè, Pobè, Benin, [6]Centre de Dépistage et de Traitement de l'Ulcère de Buruli de Zangnanado, Zangnanado, Benin, [7]Programme National de Lutte Contre la Lèpre et l'Ulcère de Buruli, Ministère de la Santé, Cotonou, Benin, [8]Department of Control of Neglected Tropical Diseases, World Health Organization, Geneva, Switzerland

Buruli ulcer (BU) is a neglected tropical disease caused by *Mycobacterium ulcerans*. Usually BU begins as a painless nodule, plaque or edema, ultimately developing into an ulcer. Striking is the high number of patients presenting with ulcers in an advanced stage. Such late presentation will complicate treatment and have long term disabilities as a consequence. The disease is mainly endemic in West Africa. Strategy for control this disease is early detection using community village volunteers. This study aims to understand the contribution of different actors in the current referral pattern in Benin, the role of the different referral systems on the stage of disease at presentation in the hospital and the diagnostic precision of Buruli ulcer. Patient information of Buruli ulcer patients that reported to one of the four BU centers in Benin between January 2008 and December 2010 was collected using the WHO/BU01 forms. Information traced from these forms were general characteristics of the patient, the results of diagnostic tests, the presence of functional limitations at start of treatment, lesion size, patient delay and the referral system. The role of the different referral systems on the stage of disease at presentation in the hospital was analyzed by a logistic regression analysis. About a quarter of the patients (26.5%) were referred to the hospital by the community health volunteers. In our data, community health volunteers seemed to refer patients more frequently in an earlier stage of disease but after adjustment for the health center, this effect could not be seen anymore. The Polymerase Chain Reaction (PCR) for IS2404 positivity rate among patients referred by the community health volunteers was not systematically lower than in patients referred by other systems. This study clarifies the role played by community health volunteers. It highlights that in Bénin, the community health volunteers are an important link in the control of BU.

1656

GREEN SYNTHESIS OF SILVER NANOPARTICLES USING FLORAL EXTRACT OF *GOMPHRENA GLOBOSA* AND ITS ANTIMICROBIAL ACTIVITY AGAINST MULTI DRUG RESISTANT BACTERIA

Kovendan Kalimuthu[1], Arokiyaraj Selvaraj[2], Murugan Kadarkarai[1], Vincent Savariar[3]

[1]Bharathiar University, Coimbatore, India, [2]National Institute of Animal Science, Suwon, Republic of Korea, [3]Loyola College, Chennai, India

Antibiotic resistance has become a major clinical and public health problem within the lifetime of most people living today. Silver nanoparticles (AgNPs) are well known biocidal substances that can be incorporated as antimicrobial agents in phar¬macology, veterinary medicine, implants, wound dressings, and topical ointments. AgNPs were also found to exhibit antimicrobial activities. The present work reports one step ecofriendly method for the synthesis of AgNPs using *Gomphrena globosa* and its antibacterial effects against drug resistant bacteria. In the present results, AgNPs was characterized by ultraviolet-visible spectroscopy, X-ray diffraction spectroscopy, Transmission electron microscopy and particle size analyzer. The synthesized particles were found to be spherical in shape and sizes ranged between 55-60 nm. Further energy-dispersive X-ray spectroscopy confirmed the presence of silver. Furthermore these green synthesized AgNPs were found to show significant antimicrobial effect against the drug resistant Methicillin resistant *Staphylococcus aureus*, cipro flaxin resistant *Escherichia coli*, and carbapenem resistant *Acetobacter baumanii.* This outcome may pave a way for using floral extract of the AgNPs a drug carrier system to cure bacterial diseases.

1657

FREQUENCY OF VIRULENCE GENOTYPES IN *ESCHERICHIA COLI* STRAINS ISOLATED FROM URINARY TRACT INFECTIONS OF MEXICAN PATIENTS

Gloria Luz Paniagua Contreras[1], Bianca Hidalgo Hernández[1], Selene Acebo Falcón[1], Eric Monroy Pérez[1], Raymundo Rodríguez Moctezuma[2], Pablo Domínguez Trejo[2], Sergio Vaca[1]

[1]Universidad Nacional Autónoma de México, Tlalnepantla, Edo. de México, Mexico, [2]Instituto Mexicano del Seguro Social, Tlalnepantla, Edo. de México, Mexico

Uropathogenic *Escherichia coli* (UPEC) is responsible for a high percentage of urinary tract infections (UTIs) worldwide, among them cystitis and pyelonephritis. The purpose of this work was to determine the frequency of the genotypes: pap (pilus associated with pyelonephritis), papGI and papGII (pilus associated with pyelonephritis GI and GII), hlyA (haemolysin), afa (afimbrial adhesin), sfa (S fimbriae), iron (iron), iuc (aerobactin), cnf (cytotoxic necrotizing factor), astA (enteroaggregative toxin), sap (she pathogenicity island marker) and set (Shigella enterotoxin 1) in a group of E. coli strains isolated from Mexican patients suffering UTIs. E. coli strains were identified by biochemical tests and by PCR amplification of 16S rRNA. Genes pap, papGI, papGII, hlyA, afa, sfa, iron, iuc, cnf, astA, sap and set were detected by multiplex PCR and by end-point PCR. Urine samples of 100 urinary tract infected patients were microbiologically analyzed. E. coli was identified in 60% of the samples (n=60). Of the E. coli strains, 48.3% (n=29) carried the set gene; 41.6% (n= 25) carried papGI; 26.6% (n=16) carried hlyA; 23.3% (n=14) carried afa; 21.6% (n=13) carried pap; 20% (n=12) carried papGII; 18.3% (n=11) carried sfa; 16.6% (n=10) carried iron; 13.3% (n=8) carried iuc; 6.6% (n=4) carried cnf1; 10% (n= 6) carried

astA and 5% (n=3) carried sap. The high frequency of the identified genes in the UPEC strains suggests that they are virulent and able to produce cystitis and/or pyelonephritis.

1658

COMMUNITY-ACQUIRED METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* INTRODUCED INTO A BRAZILIAN PUBLIC PEDIATRIC CLINIC

Mariel A. Marlow[1], Claudete Araújo Cardoso[2], Marcos Gabriel Pinheiro[3], Ivete Martins Gomes[2], Cláudia Aguiar Barros[2], Lee W. Riley[1], Fábio Aguiar-Alves[3]

[1]University of California Berkeley, Berkeley, CA, United States, [2]School of Medicine, Fluminense Federal University, Niterói, Brazil, [3]Pathology Program, Laboratory of Molecular Epidemiology and Biotechnology, Rodolfo Albino University Laboratory, Fluminense Federal University, Niterói, Brazil

Low-income communities, health care professionals, and adults in frequent contact with children are all populations known to be at higher risk for methicillin-resistant *Staphylococcus aureus* (MRSA) colonization. The objective of our investigation was to determine risk factors for colonization with MRSA as well as staphylococcal cassette chromosome *mec* (SCC*mec*) genotypes among pediatric health care workers from a public hospital in Rio de Janeiro, Brazil. We collected nasal swabs and data on potential risk factors from 178 health care workers from all pediatric sectors from January to December 2012. Swab cultures were evaluated for antimicrobial resistance against Cefoxitin and Oxacillin, and resistance was confirmed by identifying the *mecA* gene by PCR. MRSA colonization was 5.1% (n=9/178). Logistic regression analysis showed being a nurse and working in an inpatient unit as potential risk factors for MRSA colonization (adjusted OR= 11.6, 95% CI 1.2 - 110.7 and 2.7, 95% CI 0.3 - 23.7, respectively). No non-work related risk factors were identified. Four of the nine isolates were found to be SCC*mec* type IV, the genotype most commonly associated with community-acquired MRSA (CA-MRSA). Five isolates were observed to be SCC*mec* type III, with four collected from nurses of various pediatric sectors in August/September alone. We then compared these isolates with nasal MRSA isolates sampled from children within 48 hours of being admitted to the pediatric ward from December 2011 to July 2012. Of 11 CA-MRSA isolates from 92 children sampled (12.0%), ten were found to be SCC*mec* IV, including two which preceded those collected from nurses in the same sector. Given SCC*mec* type III isolates appear to have circulated among various pediatric sectors, the introduction of the more virulent CA-MRSA genotype to the pediatric inpatient ward is of high concern. Prevention in the hospital setting may also depend on interventions at the community level in low-income settings.

1659

EXTENDED-SPECTRUM BETA-LACTAMASE (ESBL)-PRODUCING *ENTEROBACTERIACEAE* ISOLATED FROM HEALTH CARE WORKERS' CELL PHONES IN FIVE PERUVIAN INTENSIVE CARE UNITS: ANTIBIOTIC RESISTANCE PATTERNS AND MOLECULAR CHARACTERIZATION

Steev Loyola[1], Luz Gutierrez[2], Gertrudis Horna[2], Jorge Osada[2], Andres G. Lescano[1], Kyle Petersen[1], Jesus Tamariz[2]

[1]U.S. Naval Medical Research Unit - 6, Lima, Peru, [2]Universidad Peruana Cayetano Heredia, Lima, Peru

Technological advances allow rapid and efficient communication through cell phones; their unsupervised use in hospital environments however, is common. Outbreaks associated with ESBL-producing (Extended-spectrum beta-lactamase) *Enterobacteriaceae* have been widely described worldwide in intensive care units (ICU), we therefore hypothesized that cell phones might represent a source of these infections in Peru. We conducted a 5-month passive surveillance in 3 Pediatric ICUs and 2 Neonatology ICUs from 3 hospitals during February to June 2012. Swabs were collected from ICU health care workers' cell phones twice monthly. Microbiological identification and resistance patterns were determined by standard methods. Suspicious ESBL-producing bacteria in the antibiogram were confirmed by the phenotypic CLSI ESBL confirmatory test. We then performed PCR for detection of blaTEM, blaSHV and blaCTX-M genes to characterize the ESBL. A total of 114 employees were enrolled, 114 devices were tested, resulting in 491 samples. Twenty-two percent (25/114) of providers phones were colonized with nosocomial pathogens. Among 105 Enterobacteriaceae isolated, 33.3% (35/105) produced ESBLs, including 18.8% (9/48) of *Enterobacter spp.,* 55.9% (19/34) of *Escherichia coli*, 26.1% (6/15) of *Klebsiella pneumoniae* and 12.5% (1/8) of *Klebsiella oxytoca*. blaCTX-M was the most prevalent ESBL. ESBLs resulted in a phenotype of Multidrug resistance: Tobramycin resistance represented 74.3% of isolates, both Ciprofloxacin and Sulfamethoxazole/Trimethropim 68.6%, Gentamicin 62.9%, Amikacin 17.1%, and Cefoxitin 5.7%. No carbapenem resistance was detected and Metallo-beta-lactamases (MBLs), Carbapenemases and AmpC beta-lactamases were not identified in isolated *Enterobacteriaceae*. Our data suggest cell phones can be an important source of ESBL spread in developing world ICUs. Methods to prevent outbreaks and transmission of these bacteria from commonly used fomites, such as cell phones, are needed.

1660

THE ROLE OF *YERSINIA PESTIS* PRESENSIBILIZATION AND GENETIC BACKGROUND IN RESISTANCE OF BLACK RATS AGAINST PLAGUE

Voahangy Andrianaivoarimanana[1], Guillaume Soubigou[2], Minoarisoa Rajerison[1], Michel Ranjalahy[1], **Ronan Jambou**[2]

[1]Institut Pasteur de Madagascar, Antananarivo, Madagascar, [2]Institut Pasteur, Paris, France

In Madagascar, plague remained endemic in the rural areas of the central highlands with the black rat, *Rattus rattus*, as the main reservoir. The occurrence of plague cases from one season to another in the same villages is questioning. One hypothesis is that part of black rats can acquire resistance to plague allowing survival of rat populations and maintenance of infected fleas and thus of the disease. We previously described resistance of field black rats living in endemic area against plague whereas those from non endemic ones remained sensitive. This study investigates whether this resistance is genetically driven or if pre immunization of rats with *Yersinia pestis* could increase survival during subsequent infections. F1 generation of black rats, originating either from plague endemic or plague free zones were obtained and challenged once or twice with *Y. pestis*. Rat survival, antibody production and gene expression were compared during the acute phase of the disease. First inoculation of a low dose of *Y. pestis* greatly increases survival of rats against a lethal dose of the bacteria. This protection of primed rats can likely be related to anti-F1 IgG. Transcriptome analysis of leukocytes five days after infection revealed that genes related to inflammation but also to apoptosis were more expressed in rats from non endemic than in those from endemic ones. In the other hand, anti-apoptotic BcL2 pathway was highly expressed in resistant rats. This suggested that rat susceptibility to infection could be driven by apoptosis of activated leukocytes. Transmission of a resistance phenotype to the F1 generation for *R. rattus* from endemic plague foci is highlited. These findings highlight the role of low transmission of bacteria in a resistance phenotype of *R. rattus* to plague. A genetic component of this resistance is also supported This study provides critical insights on the role of *R. rattus* in plague persistence in Madagascar

1661

CHARACTERIZATION OF *ACINETOBACTER* ISOLATES POSITIVE FOR IMP CARBAPENAMASE FROM PERUVIAN HOSPITALS

Nathanael D. Reynolds

U.S. Naval Medical Research Unit - 6, Lima, Peru

Carbapenem antibiotics constitute the last resort in treating multi-drug resistant *Acinetobacter*infections typically acquired during hospitalization. The effectiveness of carbapenems against *Acinetobacter* has been compromised in the last decade largely due to the emergence of carbapenem-hydrolyzing metallo-beta-lactamases (MBL). Metallo-beta-lactamases include the enzymes IMP, VIM, NDM, GIM, and SIM and account for the majority of carbapenem resistance in Gram negative bacteria. While extensively characterized in East Asia and Europe, currently there exist limited reports on MBL resistance in *Acinetobacter* infections from South America. Surveillance of hospital acquired *Acinetobacter* infections in Lima and Iquitos, Peru between March 2011 and February 2013 identified 32 suspected nosocomial isolates (11 from Lima and 21 from Iquitos) and 20 clinical and environmental isolates associated within ICU outbreaks in Lima. Four of the 52 *Acinetobacter spp.* isolates were positive for *blaIMP*, consisting of one *A. baumannii*, one *A. haemolyticus*, and two *A. junii*. Phenotypic carbapenem resistance as defined by minimum inhibition concentrations to imipenem indicates resistance in one *A. junii* and *A. haemolyticus*. Whole genome sequencing of the *blaIMP*-positive isolates identified multiple resistance genes and characterized the IMP-16 variant in the *A. baumannii* and *A. junii* isolates and IMP-18 in the *A. haemolyticus* isolate. Phylogenetic analysis indicates no relativeness between the *A. junii* isolates or any IMP-positive isolates with previously identified the IMP-positive *Acinetobacter* isolates referenced by the NCBI Whole Genome Shotgun Database.

1662

CHARACTERIZATION OF CARBAPENEMASE-POSITIVE *PSEUDOMONAS AERUGINOSA* ISOLATES IN LIMA HOSPITALS

Drake H. Tilley

U.S. Naval Medical Research Unit - 6, Lima, Peru

Pseudomonas aeruginosa is an opportunistic pathogen that accounts for ten percent of all hospital-acquired infections. Therapeutic options for treatment of *P. aeruginosa* infections are increasingly limited due to inherent antimicrobial resistance an ability to acquire new mechanisms of resistance. Recently, carbapenemases have emerged as one of the major mechanisms of acquired resistance in *P. aeruginosa* and represent a significant clinical concern due to its ability to hydrolyze the majority of beta-lactam antibiotics. The most clinically relevant and widely disseminated carbapenemases include KPC, IMP, VIM and NDM. Currently, little information has been reported on the prevalence of carbapenemase genes present in *P. aeruginosa* isolates in Peru. To begin to define carbapenemase resistance in Peru, 124 carbapenem-resistant *Pseudomonas aeruginosa* isolates were collected from nosocomial and outbreak infections from three hospitals in Lima. Antibiotic resistance to beta-lactam was identified in 18 percent (23/124) of the *P. aeruginosa,* as defined by disk diffusion assay according to the CLSI guidelines. PCR was performed on all 124 isolates to detect the carbapenemase genes *blaKPC*, *blaIMP*, *blaVIM* and *blaNDM*. From the 124 isolates, 22 (18%) *P. aeruginosa* isolates were identified as the IMP-16 variant, one (1%) isolate positive for the VIM-2 varient, and none positive for KPC or NDM, being this 23 isolates extensively drug-resistant. Finally, in order to determine genomic-relatedness, the 23 isolates with carbapenemase genes were analyzed using rep-PCR on the Diversilab system. The 23 isolates clustered into four genomically distinct groups. Interestingly, several of IMP positive isolates clones were dispersed throughout different hospitals, suggesting possible clonal spread of IMP-16 positive *P. aeruginosa* between Lima hospitals. Also, the VIM-2 positive isolate demonstrate great than 95% homology with a reference to wild type strain reflecting the capacity of carbapenem-sensitive isolates to acquire to carbapenem resistant.

1663

GENETIC CHARACTERIZATION OF RECOVERED *BACILLUS* ISOLATES FROM THE ENVIRONMENTAL SURVEILLANCE SWABS BY SEQUENCING OF *GYR*B GENE: A PUBLIC HEALTH APPROACH

Irshad M. Sulaiman, Emily Jacobs, Steven Simpson, Khalil Kerdahi

Food and Drug Administration, Atlanta, GA, United States

The primary mission of FDA is to enforce the Food, Drug and Cosmetic Act and regulate food, drug and cosmetic products. To assess adulteration in these commodities, FDA uses presence of pathogenic microorganisms in manufacturing and distribution areas as one of the regulatory action criteria and to ensure that the firm is following good manufacturing practices. Further, FDA provides guidance to achieve the said goal by establishing an environmental monitoring program in these facilities. This study was conducted to verify the effectiveness of pathogen control in a pharmaceutical compounding facility located in Southeast region of United States. A total of 28 environmental swabs were collected from several locations of a compounding company premises. The swab samples were initially examined by conventional microbiologic protocols. Of these, several swabs were found positive for the presence of rod-shaped, gram-positive bacteria, *Bacillus*. It is a diverse group of bacteria, and some of its species are human-pathogenic that can cause range of infections including ear infections, meningitis, urinary tract infections and septicemia. Species-identification of recovered *Bacillus* isolates were completed by our recently developed protocol based on nucleotide sequencing of PCR amplified *gyr*B gene products. Analysis of data confirmed four species of *Bacillus* (*B. cereus*, *B. pumilus*, *B. subtilis*, and *B. thuringiensis*) in the swabs examined. This newly developed *gyr*B-based molecular diagnostic protocol can be used as a suitable genetic marker for rapid detection of *Bacillus* in the environmental monitoring program of public health importance.

1664

PRODUCTION AND EVALUATION OF A32 KDA FRAGMENT OF THE IMMUNOGLOBULIN-LIKE B PROTEIN FROM *LEPTOSPIRA*

Hua-Wei Chen, Giulia Weissenberger, Chien-Chung Chao, Wei-Mei Ching

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

Leptospirosis is caused by spirochaetes of the genus *Leptospira*. It is considered to be the most widespread zoonotic disease in the world. Symptoms of leptospirosis are easily confused with a variety of other febrile illnesses (e.g., dengue and malaria) that require different treatment regimens. Currently, the Microscopic Agglutination Test (MAT) is the standard method for the diagnosis of leptospirosis. It is not only technically complex but also time-consuming. With the publication of the whole genome sequences of several pathogenic species of *Leptospira*, hundreds of genes encoding surface-exposed lipoproteins and outer membrane proteins have been identified as candidates for the development of rapid diagnostics of leptospirosis. An ELISA using recombinant antigens (rLipL32, rLipL41, and rLigA-Rep) for the detection of *Leptospira*-specific antibodies has been developed in our laboratory with sensitivity close to 90%. Here, we prepared a recombinant protein containing the coding region of amino acids 630-931 of LigB (rLigB-Rep). The over-expressed rLigB-Rep, which contains a six-histidine tag at the N-terminus, was primarily found in the inclusion body. The solubilized rLigB-Rep in 8 M urea was purified with a nickel column under denatured conditions. We achieved greater than 90% purity as demonstrated by SDS-PAGE. The purified rLigB-Rep was refolded by dialysis in buffer (20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1 mM EDTA) containing 6M, 4M, 2M, 1M, and no urea at 4°C. The refolded rLigB-Rep

has been shown that it was recognized by confirmed leptospirosis patient sera in western blot. These data suggest that rLigB-Rep antigen can be used to further improve the ELISA assay's sensitivity.

1665

EPIDEMIOLOGICAL PROFILE OF LEPTOSPIROSIS CASES, GUATEMALA: 2008-2013

Maria R. Lopez[1], John P. McCracken[1], Danilo Alvarez[1], David Moran[1], Rafael Ciraiz[2], Joe Bryan[3], Celia Cordon-Rosales[1]

[1]Universidad del Valle de Guatemala, Guatemala, Guatemala, [2]Ministry of Health Guatemala, Guatemala, Guatemala, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States

Leptospirosis is a widespread zoonosis with a higher incidence in countries with humid subtropical or tropical climates. *Leptospira* are usually transmitted to humans through soil or water contaminated with infected urine from mammal hosts (mainly rodents). Outbreaks are associated with flooding, agriculture, as well as recreational, sporting and military activities. In Guatemala, there are reports of cases in humans and animals but the epidemiology of leptospirosis is unknown. Active surveillance for suspected leptospirosis cases was conducted during 2008-2013 among patients with acute febrile illness (AFI) attending the Cuilapa National Hospital (932 M) or Nueva Santa Rosa ambulatory facilities (1005 M) in the Department of Santa Rosa, in southern Guatemala. Leptospirosis is typically an undifferentiated AFI but suspected cases are seldom confirmed with laboratory diagnosis. In this study, AFI was defined as self-reported fever or measured temperature ≥38°C that began <7 days before presentation with no other diagnosis (e.g. pneumonia or diarrhea). Blood samples were taken and tested for IgM anti-*Leptospira* by enzyme-linked immunosorbent assay. Of 553 patients studied (396 hospitalized cases and 157 ambulatory cases), 25 (6%) hospitalized patients were positive while 8 (5%) ambulatory patients were positive. The median of age (IQR) of cases was older (22 years (16-34)) than the leptospirosis negative cases (16 (7-29)). Most cases (79%) were between 10-39 years and 53% were male. The majority of the patients presented with nonspecific signs, such as headache (91%), nausea (85%), myalgia (81%), vomiting (79%), arthralgia (67%), and hemorrhages (12%). During 2008-2013, 22 cases (67%) were detected in the rainy season (May-Oct). Cases were higher in 2010, the year with the most rain in a decade due to tropical storm Agatha, with 18 (55%) cases detected and 14 (78%) in the rainy season. Given endemic nature of leptosporosis in Guatemala and Central America, efforts on prevention and control should focus on these events and the greater risk among adults.

1666

EXTENDED EMPIRICAL TREATMENT CONTRIBUTES TO CHANGES IN BACTERIAL RESISTANCE PROFILE

Wellington F. Rodrigues[1], Camila B. Miguel[1], Ana Paula O. Nogueira[2], Carlo J. Oliveira[1], Marcelo C. Araújo[1], Javier E. Lazo-Chica[1]

[1]Universidade Federal do Triângulo Mineiro, Uberaba, Brazil, [2]Universidade Federal de Uberlândia, Uberlândia, Brazil

Extra intestinal infections acquired in the community with *Escherichia coli (E. coli*) showed in the United States, a rate of frequency of six million to 8 million cases of uncomplicated cystitis and 127,500 cases of sepsis per year. Empirical treatment has been associated with the emergence of bacterial resistance. although some countries are concerned to switch drugs used to treatment infectious, others keep the same treatment (for treatment empirical) over a long period This study aims to assess the sensitivity and resistance profile of E. coli across the different drugs used for treatment. A cross-sectional and retrospective study (January 2008 to December 20013) at a public hospital, university, belonging at System of Health of Minas Gerais/Brazil (empirical treatment = fluoroquinolones), was considered only in patients ambulatory that presented symptoms (dysuria). The antibiotics tested were as follows (potency in µg/disc): ampicilin + sulbactam (10/10), cephalothin (30),ciprofloxacin (5), norfloxacin (10) e nitrofurantoin (300) (standard disc diffusion method as per CLSI guidelines using discs of standard potency. Furthermore the costs of different classes of antibiotics were compared. Statistical analysis was performed using the program "Prism" from Graphpad. The results showed that all antibiotics tested here are effective ($p < 0.05$). However there was an increase in the sensitivity of nitrofurantoin in relation to other antibiotics with a decrease of the resistance of the same antibiotic in compared with others ($p < 0.05$), and a variation in the sensitivity and resistance among the fluoquinolonas with the ampicillin more inhibited beta-lactamase. There was also a significant difference in cost, and showed are more affordable the nitrofurantoin ($p < 0.05$), followed by fluoroquinolones, beta-lactamase inhibitor and cefalotinas. In conclusion, this work shows that should be considered an alternation of treatments for infection by E. coli, thus favoring the control of bacterial resistance and the cost effective to the population.

1667

ANTIBIOTIC RESISTANCE PATTERNS OF *ENTEROBACTER* SPECIES ISOLATED FROM CHILDREN WITH CYSTITIS IN IRAQ

Maitham G. Yousif

Al-Qadysia University, Dewania, Iraq

Awareness of antibiotic sensitivity patterns among gram negative bacterial pathogens is clinically important not only to empirically guide therapy but also to monitor the emergence of new drug resistant strains. We report here a survey of antibiotic resistance in Enterobacter species cultured from children with cystitis in Iraq. We collected 1,474 urine cultures from children aged 1-7 years treated at Al-Hakeem Hospital in Najaf governate, Iraq between January and September of 2010. Enterobacter species were identified in 3.9% of cultures (n=57), and were found more commonly in females (68.4%) than males (13.6%). Cultures positive for *Enterobacter* occurred most frequently in February followed by July. The following seven antibiotics were tested on isolates: cephalexin, cefotaxime, ceftriaxone, gentamicin, nalidixic acid, ciprofloxacin and amikacin. Antibiotic resistance variations were measured monthly and appeared to have a seasonal dependence. In January *Enterobacter* isolates were strongly resistant to cephalexin, in February to cefotaxime, in March to ceftriaxone, cephalexin and gentamycin, and in April to cefotaxime and nalidixic acid. In July isolates showed no resistance to amikacin and low resistance to ciprofloxacin, while in August and September strong resistance to cephalexin. Identification of factors which lead to an apparent seasonal variations in antibiotic resistance patterns among *Enterobacter* will require further study that includes careful evaluation of demographic and therapeutic histories of patients from whom *Enterobacter* strains are isolated, speciation of these isolates and collection of larger numbers of isolates over longer periods of time in order to control for sampling variability.

1668

SIGNIFICANT DIFFERENCES IN ULTRASOUND FINDINGS BETWEEN MALNOURISHED AND NON-MALNOURISHED SCHOOLCHILDREN IN MADAGASCAR

Maria Teresa Giordani[1], Daniela Sardei[2], Indi Trehan[3], Tom Heller[4], Jacopo Nicoletti[5], Enrico Brunetti[5]

[1]San Bortolo Hospital, Vicenza, Italy, [2]Pediatrician, Carrè, Vicenza, Italy, [3]Washington University, St. Louis, MO, United States, [4]Klinikum Muenchen-Perlach, Muenchen, Germany, [5]University of Pavia, IRCCS S. Matteo Hospital Foundation, Pavia, Italy

Childhood malnutrition contributes to high mortality rates and decreased educational and adult capacity among survivors. Not just closely linked to an increasing burden of infectious diseases, it is also increasingly recognized as a cause of chronic morbidity in later life. We explored the utility of bedside ultrasound in identifying specific pathologic findings in malnourished children in Madagascar, with the aim of improving the

management of co-morbidities. 83 children with stunting and/or severe acute malnutrition underwent bedside abdominal sonography and compared with 76 non-malnourished children. Liver and spleen size, liver echogenicity, intraluminal bowel evidence of massive helminthic infections, enlargement of abdominal lymph nodes, thickening of the gallbladder wall, and other pathologic findings were assessed. Malnourished children had hepatosplenomegaly (36% vs 18%) and fatty liver (41% vs 18%) more frequently than non-malnourished children. Hepatosplenomegaly was more common in the Antaimoro area, where malaria and sickle-cell anemia are more prevalent. Evidence of intestinal helminth infections were common in both groups, but in non-malnourished children were mostly associated with fever and acute diarrhea. Other pathologic findings were present In 17 malnourished children (20%) compared with 8% of non-malnourished children. Preliminary results of this study suggest that ultrasound evaluation of malnourished children is feasible and can be aid in identifying co-morbidities. The high rates of fatty liver infiltration particularly deserves more attention, as a possible marker for the development of metabolic diseases and liver fibrosis in adulthood.

1669

LEPROSY, A MIMICKER OF OTHER DISEASES IN A DEVELOPED COUNTRY

Rone Lin[1], Winnie Ooi[2], Samuel Moschella[2], Stephanie Burns[2]

[1]Tufts Medical Center, Boston, MA, United States, [2]Lahey Hospital and Medical Center, Burlington, MA, United States

Leprosy (Hansen's disease) is an uncommon disease in the U.S. and therefore unfamiliar to many health-care providers. This disease primarily occurs in immigrants from countries with higher endemicity such as India and Brazil. Initial misdiagnosis can lead to significant delay in therapy, morbidity and loss of function. Here we reviewed our database of 172 patients with leprosy, who were evaluated and treated in our Hansen's disease public health satellite clinic from 1998 to 2014. There were 18 patients (10%) with prior misdiagnoses; eleven of them initially presented with skin rash, six with neuropathy and one with rheumatologic symptoms. Skin rash from Hansen's disease can be difficult to distinguish from more common dermatologic conditions such as fungal skin infection, allergic dermatitis and cutaneous sarcoidosis. Idiopathic mono-neuropathies with wrist or foot drops, as well as mononeuritis multiplex, were among the common misdiagnoses for patients with leprous neuritis. Leprosy can also mimic rheumatoid arthritis with solely joint symptoms without initial involvement of skin or nerve. In our review, the diagnosis could be delayed for a significant amount of time, for even up to 10 years in 2 cases. This led to irreversible loss of neurologic function (foot/wrist drops), neuropathic ulcers and osteomyelitis. We will present 3 illustrative cases: one of leprous neuritis misdiagnosed as mononeuritis multiplex from sarcoidosis, a second of polyarthritis from leprosy mimicking rheumatoid arthritis, and lastly, a case of borderline tuberculoid leprosy with skin rash thought to be from cutaneous sarcoidosis based on initial evaluation of a skin biopsy. To summarize, it is of crucial importance to include leprosy in the differential diagnoses for patients with chronic rash, neuropathy or joint symptoms in the appropriate epidemiologic setting, because early recognition and treatment can prevent progression of the disease and its morbidities.

1670

TWO FATAL CASES OF MELIOIDOSIS ON THE THAI-MYANMAR BORDER

Miriam B. Torchinsky[1], Cindy S. Chu[2], Clare Ling[2], Francois Nosten[2]

[1]University of British Columbia, Vancouver, BC, Canada, [2]Shoklo Malaria Research Unit, Mae Sot, Thailand

Melioidosis, caused by the gram-negative environmental bacterium *Burkholderia pseudomallei*, is an infectious disease of clinical importance in endemic areas, and is associated with a high case-rate fatality in humans and animals. Once considered an esoteric tropical disease, research on *B. pseudomallei* has gained prominence due to its potential for epidemic spread, increasing numbers of case reports from non-endemic regions, and classification by the United States as a potential bioterrorism agent. Lack of awareness among physicians, along with a wide variability in disease manifestations, contributes to underdiagnosis and delayed treatment, and also confounds accurate assessment of global prevalence. Although melioidosis is endemic in Northern Australia and parts of Southeast Asia, there are no published reports from the Thai-Myanmar border. Here we report the first two documented cases of fatal melioidosis in this region. The discussion of cases in as-yet-unrecognized foci of disease is of great public health importance and may help to better elucidate environmental and host determinants of infection. Our study highlights the need to both increase clinical awareness of melioidosis on the Thai-Myanmar border, and to better assess the true burden of disease in the region through improved case detection and rigorous *B. pseudomallei* prevalence studies.

1671

HISTOPLASMOSIS IN OREGON EX ECUADOR

Jon E. Lutz[1], Jonathan Brewer[1], John Blizzard[2]

[1]Bend Memorial Clinic and St. Charles Medical Center, Bend, OR, United States, [2]Department of Cardiothoracic Surgery, Bend, OR, United States

A 41 year old river raft guide returned from a 2 week long rafting trip in a jungle region of Ecuador on March 30, 2012. He had received appropriate travel vaccinations and had taken mefloquine for malaria prophylaxis. Shortly after his return he developed fever to 103 F range, chills, sweats, headache, myalgias, arthralgias and fatigue. He self-medicated with amoxicillin and subsequently ciprofloxacin was prescribed by a physician. On examination on April 9th he was afebrile, had some shotty cervical and inguinal lymphadenopathy but otherwise had a normal physical examination including that of the lungs. Initial laboratory investigations including malaria smears, blood cultures and stool studies were unrevealing. A chest x-ray had evidence of a right sided infilatrate. Ceftriaxone and doxycycline were given but fevers persisted. A CT scan of the chest was carried out and revealed extensive mediastinal and hilar lymphadenopathy and too numerous to count lung nodules. Fiberoptic bronchoscopy was nondiagnostic. On April 20th therapy with ketoconazole was initiated to treat possible paracoccidioidomycosis. On April 24th, histoplasmosis serologies were reported positive. He was started on liposomal amphotericin B. On April 25th he underwent a minithoracotomy for definite diagnosis as there was concern about possible coexisting malignancy. Pathology and intraoperative cultures were consistent with histoplasmosis. He subsequently completed a 3 month course of itraconazole and did well. We herein discuss briefly travel-associated histoplasmosis and the current recommendations for drug therapy of severe primary histoplasmosis infection.

1672

DESCRIPTION OF DENGUE-RELATED HOSPITALIZATION AND DISEASE SEVERITY FROM AN ENHANCED DENGUE SURVEILLANCE SYSTEM IN PUERTO RICO

Janice Pérez-Padilla[1], Eric González[2], Aidsa Rivera-Sánchez[1], Brenda Torres-Velásquez[1], Juan P. Torres[2], Luisa Alvarado[2], Kay M. Tomashek[1]

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

Dengue is an acute febrile illness (AFI) that is endemic in Puerto Rico. The clinical spectrum of dengue ranges from mild AFI to a life-threatening illness. Although timely identification of dengue patients can reduce medical complications and mortality, this complicated by clinical manifestations that overlap with other AFI. To identify early clinical features that can be used as predictors for severe dengue, we evaluated the clinical course of laboratory-positive (i.e., DENV nucleic acid detected by RT-PCR or anti-DENV IgM antibody detected by ELISA) dengue patients

enrolled in the Sentinel Enhanced Dengue Surveillance System (SEDSS) site located in Ponce, Puerto Rico. Patients were those presenting with AFI during May 7, 2012 to May 6, 2013 that were hospitalized (n = 262) or were out-patients that returned for follow-up evaluation (n = 120). Of all 382 patients, there were no significant differences in age or sex between hospitalized and non-hospitalized patients. Admitted patients sought care later than non-hospitalized patients (mean day of presentation = 4 vs.2 days), and had a mean hospital stay of 4 days. Clinical findings associated with hospitalization were anorexia ($p = 0.002$), diarrhea ($p = 0.021$) and dengue warning signs of persistent vomiting ($p < 0.001$), abdominal pain ($p < 0.001$) and bleeding ($p = 0.013$). Laboratory findings at presentation associated with hospitalization were leukopenia ($p = 0.021$) and thrombocytopenia ($p < 0.001$). Mean platelet count was significantly different between hospitalized and non-hospitalized patients (mean = 81,000 vs. 151,000) ($p < 0.001$). Patients that presented 4-7 days after illness onset had greater odds of having thrombocytopenia (OR = 2.18; CI: 1.24-3.83) or elevated liver transaminases (OR = 4.74; CI: 1.53-19.45). Enhanced dengue surveillance revealed that hospitalized dengue patients presenting late for clinical care were more likely to present with dengue warning signs, and were hospitalized more frequently. Further analysis will assess correlation between early presentation and ultimate disease severity.

1673

ACCESS MATTERS: AFRICAN-BORN U.S. MILITARY TRAVELERS UTILIZE TRAVEL CARE AT HIGH RATES

Kristina St. Clair[1], Anthony R. Artino[2], Patrick W. Hickey[2]

[1]Naval Medical Center Portsmouth, Portsmouth, VA, United States, [2]Uniformed Services University, Bethesda, MD, United States

Immigrant travelers that visit friends and relatives (VFRs) are less likely to have a pre-travel health encounter and more likely to experience serious illness. Immigrants comprise more than 5% of active duty service members in the United States Department of Defense and have free access to travel medicine care, yet their travel health seeking behaviors are unknown. 351 African-born service members and 470 U.S.-born comparators completed an Internet-based survey to assess pre-travel health care utilization, perceived potential barriers, and health outcomes. While overall use of travel medicine services was equivalent if official duty was included ($p=0.94$), when traveling on leave status (not on official duty) African-born service members were more likely than U.S.-born to see a physician, 65% vs. 35% ($p<0.001$) prior to their most recent travel to a low, low-middle or upper-middle income country. This persisted when stratified by malaria risk at destination with African-born service member VFRs reporting pre-travel health care more than American-born comparators, 65% vs. 45% ($p < 0.001$) Both African-born and American-born service members reported easy access to medical care. African-born military service members perceive less risk of illness when traveling to Africa compared to American born travelers ($p<0.001$) yet, somewhat paradoxically, place more importance on pre-travel medical services ($p = 0.007$). African-born service members are more willing to self-diagnose and treat illnesses such as malaria ($p<0.001$) and rely on locally purchased medications ($p<0.001$). There was no difference in reported adherence to malaria chemoprophylaxis. This United States Military Health System study revealed data that opposes previous civilian studies: African-born VFRs in the military sought pre-travel health care more often than their U.S.-born counterparts. Access to care and positive beliefs about the benefits of travel medicine services contribute to this finding. These findings have implications for the role of national health-care reform and community engagement programs. Disclaimer: The Views expressed are those of the author(s) and do not necessarily reflect the official views of the Uniformed Services University of the Health Sciences, the U.S. Army, or the Department of Defense.

1674

SURVEILLANCE FOR ARTEMISININ, QUININE, AND MALARONE RESISTANCE AMONG IMPORTED *PLASMODIUM FALCIPARUM* MALARIA - CALGARY, CANADA (2013-14)

Asongna Folefoc, Abu N. Mohon, Lydia Lee, **Dylan R. Pillai**

University of Calgary, Calgary, AB, Canada

Calgary has seen an increase in imported malaria cases in recent years with a large proportion coming from sub-Saharan Africa where *Plasmodium falciparum* is hyper-endemic. Surveillance for emerging resistance to antimalarial drugs such as Artemisinin, Quinine, and Malarone is essential in returning travelers. Artemisinin resistance has been reported in Cambodia and mutations within the K13-propeller gene associated with resistance to this drug. Mutations in the cytochrome B (cytB) are linked to Malarone resistance. In this study, we prospectively determined the susceptibility and resistance genotype of imported *P. falciparum* malaria to Calgary from April, 2013 to April, 2014. Travelers were mostly male (n=12, 67%) VFR (n=17, 94%) with mean age of 35.3 years destined for sub-Saharan Africa (n=17, 94%) predominantly West Africa (n=11, 61%). Malarone (oral) was the commonest treatment option (n=11, 61%) followed by artesunate (n=3, 17%) and quinine (n=3, 17%). Positive malaria samples from patients (n=18) were tested with a standardized panel of antimalarials using the ELISA-based HRP2 *ex vivo* drug sensitivity protocol developed by WWARN. DNA was extracted from patient EDTA blood samples (n=18) and primers flanking the K-13 propeller gene and cyt B were used for PCR amplification of this gene. PCR products were bidirectionally sequenced and analyzed for mutations. Our ex-vivo results showed IC_{50} values of 17.36 ± 11.92nM, 4.34 ± 2.34 nM, 4.06 ± 1.66 nM, 4.00 ± 1.39 nM for Artemisinin, Artesunate, Artemether and Dihydroartemisinin, respectively; mean IC_{50}s of 39.04 ± 15.73 nM, 16.33 ± 4.36 nM, 80.44 ± 25.75 nM, 17.23 ± 3.65 nM and 127.38 ± 36.97nM for Chloroquine, Mefloquine, Quinine, Amodiaquine and Piperaquine respectively; and mean IC50 = 27.2 ± 22.26 for Atovaquone. Analysis of the K-13 propeller and CytB gene showed that all imported malaria were wild type to date. Our results confirm that imported *P. falciparum* malaria to Calgary from sub-Saharan Africa remains wild-type at key resistant loci (K13 and cyt B) and susceptible to Artemisinin, Malarone and quinine the commonest treatment options when tested *ex vivo*.

1675

A SURVEY ON KNOWLEDGE, ATTITUDES AND PRACTICES AMONG INTERNATIONAL TRAVELERS IN UGANDA

Zain Chagla[1], Palmyra Immordino[2], Claire Webb[2], Ronald M. Mbwasi[2], Christopher Sanford[3]

[1]McMaster University, Hamilton, ON, Canada, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]University of Washington, Seattle, WA, United States

In an increasingly international environment, global travel rates are increasing. However, significant risk exists amongst travelers. Counseling against preventable morbidity and mortality, such as transit related injuries, vaccine preventable diseases, violent crimes, and tropical illnesses, is essential for international travelers. The majority of data around traveler attitudes and counseling is based on pre-travel assessments, or in evaluation of returned ill travelers, but is biased towards those with health seeking behaviors. This study was designed to look at attitudes and practices amongst international travelers while actively travelling. The study took place Jinja, Uganda, a popular tourist destination near Kampala known for white-water rafting and the Nile River. Participants were recruited at tourist locales in Jinja, and a semi-structured questionnaire was administered to tourists after obtaining voluntary informed consent. A total of 153 travelers were surveyed. The majority was female, with average age 31 years, and predominantly from the USA, Australia, and the Netherlands. Most participants had received pre-travel advice through a travel clinic (64.0%) or a general practitioner (25.4%), and others cited the Internet, friends, and relatives as sources of information. Participants

endorsed malaria (94.7%), vaccines (92.1%), and diarrhea (59.5%) were the most important pre-travel counseling items, but few mentioned traffic accidents (15.0%) or sexually transmitted infections (13.7%). Malaria prophylaxis was prescribed to the majority (80.1%), but only three quarters of those people took prophylaxis, with adherence issues attributed to side effects or a lengthy duration of stay. Finally, when asked about health and safety issues experienced during the trip, nearly a third had encountered an issue, most of which either related to gastroenteritis, malaria, or traffic accidents. Although many travelers seek medical care prior to departure, counseling regarding non-infectious issues such as road traffic accidents, personal safety, and risk behaviors are lacking, despite these being a major cause of morbidity and mortality. Integration of this information into Internet resources and clinical practice may help to decrease mortality amongst global travelers.

1676

FACTORS ASSOCIATED WITH MORTALITY BY DENGUE

Elizabeth Borrero

Fundación Santa Fe de Bogotá, Bogotá, Colombia

Dengue is a public health priority in Colombia due to the significant increase in the number of cases from 26817 in 2008 to 57589 in 2013. In 2010 a 183% increase was noted in respect to 2009 and 9393 cases of severe dengue was reported and 217 confirmed deaths. A case-control study was conducted in the departments of Cundinamarca, Norte Santander, Santander, Cauca Valley and Meta to identify the clinical factors associated with mortality in patients with severe dengue in hospitals of level II and III attention between the periods 2009-2013. A case was considered to be death by dengue and a control pertained to survivors, all were confirmed through IgM or RT-PCR. The sample size was 50 cases and 150 controls. For the analysis logistic regression was utilized. 42% (63) of the patients originated from Cauca Valley, 24% (36) Meta, 18% (27) North of Santander, 10% (15) Santander and 6% (9) Cundinamarca. 60% (90) were less than 16 years and 52% were women. The factors associated independently with mortality were: comorbidities OR 3,18 (IC 95%: 1,33; 7,60), social risk OR 3,33 (IC 95%: 1,21; 9,17), tachycardia OR 8,94 (IC 95%: 2,57; 31,05), tachypnea OR 2,71 (IC 95%: 1,16; 6,28), altered state of consciousness OR 12,09 (IC 95%: 2,72; 53.70), respiratory difficulty OR 5,61 (IC 95%: 2,24; 14,06), pleural effusion OR 2,85 (IC 95%: 1,33; 6,11), main organ damage OR 3,14 (IC 95%: 1,29; 7,64), severe bleeding OR 2,66 (IC 95%: 1,19; 5,95), and previous consultation OR 2,68 (IC 95%: 1,16; 6,19). In the multivariate analysis the factors associated with increased death were: social risk social OR 9.88 (IC 95%: 1,26; 77,11), altered state of consciousness OR 11.48 (IC 95%: 1,34, 97,93), respiratory difficulty OR 9.84 (IC (95%: 1,96; 49,36) main organ damage OR 9.55 (IC 95%: 1,77; 51,41) and severe bleeding OR 8,08 (IC 95%: 1,95; 33,66). Patients with these clinical characteristics should be hospitalized for extended observation and opportune treatment to avoid death.

1677

ATORVASTATIN FOR THE TREATMENT OF RHEUMATOID ARTHRITIS IN IRAQ

Sami Salman[1], Samer Shuker[2]

[1]Rheumatology Unit, College of Medicine, Baghdad University, Baghdad, Iraq, [2]Pharmacology Unit, College of Medicine, Baghdad University, Baghdad, Iraq

Rheumatoid arthritis (RA) is a common chronic inflammatory and disabling disorder that is characterized by synovitis, articular destruction, and many systemic extra articular features. In addition, RA is associated with both morbidity and mortality due to accelerated atherosclerosis and risk of increased cardiovascular disease. Atorvastatin is well known anti dyslipidemic agent that is considered to have potential anti-inflammatory and immune modulatory functions in patients with RA. To explore the possible anti-inflammatory effects of Atorvastain in patients with RA, we designed a study to evaluate the effect of atorvastatin compared to therapy with two more standards RA medications, methotrexate (MTX) and etanercept (EPT). The study group included Iraqi patients with moderate to highly active RA. A double blind, randomized, placebo - controlled clinical trial was conducted in which 100 RA male and female patients were enrolled from a group who were already on MTX or EPT for at least 1 month. This pool of subjects was divided into two groups, one to receive 20 mg atorvastatin tablet and the other to receive placebo capsules for three consecutive months. This study revealed first that only 49 patients completed the 3 months trial, 25 patients in atorvastatin and 24 patients in placebo group. All patients were clinically evaluated by measuring swollen joint count (SJC), tender joint count (TJC), visual analogue scale (VAS) and disease activity score (DAS28). Blood samples of all subjects patients were evaluated for erythrocyte sedimentation rate (ESR), C reactive protein (CRP) at baseline, monthly and at the end of the study. RA patients undergoing 20 mg atorvastatin treatment showed a significant ($P < 0.05$) reduction in CRP, SJC and TJC compared to those who received placebo. In addition, atorvastatin treatment groups trended toward reduced ESR, VAS, and DAS28, but these differences did not achieve statistical significance ($p > 0.05$). In conclusion we believe that 20 mg atorvastatin is a safe and well-tolerated drug that has modest anti-inflammatory effect in patients with moderate to severe active RA.

1678

GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY AND DENGUE IN SINGAPOREAN MALES: A CASE-CONTROL STUDY

Tun-Linn Thein, Linda K. Lee, Joshua G. Wong, David C. Lye, Yee-Sin Leo

Tan Tock Seng Hospital, Singapore, Singapore

Clinical presentations of dengue infection range from asymptomatic, non-severe to severe disease. We aim to test the hypothesis that patients with glucose-6-phosphate dehydrogenase (G6PD) enzyme deficiency may present with severe disease and hemolysis. We analyzed a cohort of adult dengue patients treated at Tan Tock Seng Hospital, Singapore from January 2005 to December 2008. Dengue infection was confirmed by positive polymerase chain reaction or dengue serology with World Health Organization (WHO) probable dengue definition. Singaporean males with documented G6PD status were defined as cases. For each case, three controls were selected by matching citizenship and year of infection. Hemolysis was defined as low hemoglobin concurrent with low serum haptoglobin, or high reticulocyte or lactate dehydrogenase or bilirubin. Dengue hemorrhagic fever and severe dengue were classified according to WHO 1997 and 2009 dengue guidelines. Compared with cases (n=30), controls (n=120) were significantly younger (median 26 vs. 35 years, p0.05). During their clinical course, cases had significantly higher rates of jaundice (10% vs. 1%, P<0.05), serum bilirubin (median 27 vs. 10 mmol/L, p<0.001), aspartate transaminase (median 148 vs. 91 U/L, p<0.05), and lower hematocrit (45% vs. 46%, p<0.001), haemoglobin level (13 vs. 14 mg/dL, p<0.001). There was no difference in rates of dengue hemorrhagic fever (23% vs. 22%, p>0.05). However, cases had higher tendency to develop severe dengue and hemolysis than controls ([23% vs. 12%] and [14.29% vs. 2.7%] respectively) although the difference was not significant (p>0.05). The two groups had similar rates of blood and platelet transfusions, intravenous fluid and length of hospitalization (p>0.05). The observed differences should be prospectively validated in larger cohorts and in different populations.

1679

A RANDOMIZED, DOUBLE BLIND, CLINICAL TRIAL OF TWO DOSE REGIMENS OF VINS POLYVALENT ANTIVENOM FOR THE TREATMENT OF SNAKEBITE WITH NEUROTOXIC ENVENOMATION IN NEPAL

Emilie Alirol[1], Sanjib K. Sharma[2], Anup Ghimire[2], Antoine Poncet[1], Christophe Combescure[1], Khaled Mostaguir[1], Chabilal Thapa[3], Vijaya Paudel[4], Robert W. Taylor[5], Deb Pandey[6], David A. Warrell[7], Ulrich Kuch[6], **François Chappuis**[1]

[1]Geneva University Hospital, Geneva, Switzerland, [2]B.P. Koirala Institute of Health Sciences, Dharan, Nepal, [3]Dumkauli Primary Health Care Centre, Nawalparasi, Nepal, [4]Bharatpur Hospital, Chitwan, Nepal, [5]Mahidol Oxford Research Unit, Bangkok, Thailand, [6]Forschungszentrum Biodiversität und Klima (Bio+C), Frankfurt am Main, Germany, [7]University of Oxford, Oxford, United Kingdom

Snakebite is an important medical emergency in rural Nepal. Although accurate figures are lacking, over 20 000 cases are recorded annually, with a case fatality rate close to 15%. Important variations exist between hospitals in the management and outcome of snakebite envenomation. In particular, striking disparities are observed in the dosage of antivenom, reflecting poor adherence to the complex national protocol. Although clinical studies have been conducted on viperid snake envenomation, well-designed dose-finding studies are almost non-existent for elapid envenomation. The purpose of this trial was to compare the efficacy and safety of two antivenom dosing schemes in the treatment of neurotoxic envenomation. The trial was conducted between May 2011 and March 2013 in 3 health facilities of southern Nepal. 157 patients presenting with signs of neurotoxic envenomation were randomized either to a high initial dose regimen (intervention) or to the low initial dose regimen as recommended by the Nepalese national protocol (control). The primary composite outcome included death, requirement for manual ventilation and worsening of neurotoxicity. Secondary outcomes included time to recovery, occurrence of adverse reactions, and cost. There was no statistically significant difference between arms in the proportion of patients reaching the primary endpoint (control 48.7% vs intervention 38.5%, p=0.264). No differences were observed in the analysis of safety outcomes. In 51 patients the snake species could be identified. 29 had been bitten by cobras (*Naja spp*) and 22 by kraits (*Bungarus spp*.). Those bitten by kraits experienced more primary outcomes (68.2% versus 27.1%, p=0.004), and recovered less often (40.9% vs 96.5%, p<0.001) or more slowly (mean time 18 hours vs 5 hours, p<0.001) than did patients bitten by cobras. These findings suggest that there is no difference in efficacy and safety between low and high initial dose of antivenom for neurotoxic snakebite, and that envenomation due to krait bites is less responsive to antivenom than that following cobra bites.

1680

FACTORS ASSOCIATED WITH COMPLETE ROUTINE IMMUNIZATION STATUS OF CHILDREN 12-23 MONTHS IN RURAL AREAS OF OSUN STATE - SOUTHWESTERN NIGERIA

Elizabeth B. Adedire[1], Ikeoluwapo O. Ajayi[2], Olufemi Ajumobi[1]

[1]Nigerian Field Epidemiology and Laboratory Training Program, FCT Abuja, Nigeria, [2]University of Ibadan, Ibadan, Oyo State, Nigeria

Immunization is a cost-effective public health intervention to reduce morbidity and mortality associated with infectious disease. Incomplete immunization status especially in rural areas has led to a high burden of VPD in children. The Nigerian Demographic and Health Survey, 2008 showed that only 57.8% of children had received all recommended vaccines in Osun State far below WHO target of 80%. We conducted this study to identify the factors associated with complete immunization status of children in rural areas of Osun State. A total of 750 mothers of children aged 12-23 months were interviewed, using the WHO 30 cluster sampling technique. We collected data on socio-demographic characteristics, history of vaccination and factors associated with immunization status using semi-structured questionnaire, vaccination cards were also reviewed. We defined a completely immunized child as a child who had received one dose of BCG, three doses of oral polio vaccine, three doses of Diptheria-Pertusis-Tetanus vaccine and one dose of measles vaccine by 12 months of age. Bivariate and multivariate data analysis was performed using Epi-info software. Of the 750 mothers interviewed, (36.6%) were fully immunized. Children of mothers with poor knowledge on immunization were less likely to be fully immunized (Odds ratio (OR) =0.55, 95% CI=0.23-0.51). Children whose mothers possessed primary or no formal education were less likely to be fully immunized compared to children of mothers with at least a secondary level education (OR=0.50, 95%CI=0.34-0.73). Children delivered at health facilities were more likely to be fully immunized (OR=1.81, 1.21-2.69). The major determinants of complete immunization status were knowledge level, maternal educational status and place of birth of the children. Raising the level of knowledge and increasing maternal literacy level as well as encouraging health facility births are essential to improve immunization coverage in these rural communities.

1681

VARIATIONS IN PRESENTATION OF *ERYTHEMA NODOSUM LEPROSUM*: REPORT OF THREE CASES SEEN AT A U.S. HANSEN'S DISEASE CLINIC

Kristoffer E. Leon[1], Robert W. McDonald[2], Anandi N. Sheth[2], Jessica K. Fairley[2]

[1]Emory University, Atlanta, GA, United States, [2]Emory University School of Medicine, Atlanta, GA, United States

Hansen's disease (leprosy) remains the leading infectious cause of disability, with 250,000 new cases reported globally yearly. Erythema nodosum leprosum (ENL) reactions, or Type 2 reactions, are humoral responses of the immune system that can cause systemic illness, including fever, skin lesions, joint pains and neuritis. ENL reactions occur in patients on the lepromatous end of the spectrum, classified by the Ridley-Jopling system. In non-endemic countries, Hansen's disease (HD) remains rare and often underrecognized with the literature lacking in clinical descriptions of leprosy complications in the United States. To fill this gap, we report three patients with lepromatous leprosy who were seen at a HD clinic in Atlanta, GA with complicated ENL reactions within the last three years. The first patient was a 33-year-old Bangladeshi woman who presented with high fever, abdominal pain, and arthralgias. She lacked the distinctive skin lesions usually seen in ENL, but was incidentally found to have splenic lesions. She responded well to prednisone and was able to be weaned off after 6 months. Second, a 42-year-old Vietnamese man initially presented with classic ENL lesions, fevers, and lymphadenopathy that progressed in severity despite increasing doses of corticosteroids. He eventually was admitted to the intensive care unit with a severe systemic inflammatory response syndrome. He was subsequently started on thalidomide without recurrences. The last patient was a 68-year-old U.S.-born man, who displayed symptoms representative of both Type 1 and Type 2 reactions as his initial presentation of HD. These included joint pain, severe extremity swelling, skin nodules and a progressive neuropathy. He had been misdiagnosed with a seronegative arthritis prior to this presentation. While all three cases reported are ENL, the differences of clinical courses and presentations highlight the complexity of the disease and the need for increased awareness of unique manifestations of lepromatous leprosy.

1682

SEROLOGICAL STUDY OF ANTIBODIES ANTI-*TOXOCARA CANIS* EVALUATED BY ELISA AND WESTERN BLOT IN PEDIATRIC PATIENTS WITH CRYPTOGENIC EPILEPSY

Maria de Lourdes Caballero-Garcia[1], Jocelyn Simon-Salvador[1], Juan Hernandez-Aguilar[1], Benjamin Nogueda-Torres[2], Enedina Jiménez-Cardoso[1]

[1]Hospital Infantil de Mexico Federico Gomez, Mexico City, Mexico, [2]Escuela Nacional de Ciencias Biologicas, Mexico City, Mexico

It has been suggested a correlation between infection by *Toxocara canis* and epilepsy and it is thought that epileptic seizures can be derived from the immune response generated by the presence of the parasite or direct lesions that can cause in the brain. The objective was to determine antibody anti-*T. canis* against antigen of the parasite through the technique of ELISA and Western blot in pediatric patients with cryptogenic epilepsy attending outpatient consultations in the area of Neurology of the Children Hospital of México Federico Gomez (HIMFG). We analyzed 111 patients from 6 to 16 years of age with confirmed diagnosis of epilepsy with clinical and epidemiological background who attend the external consultation of Neurology of the HIMFG. We analyzed the presence of antibodies anti-*T. canis* by ELISA using excretion-secretion antigens obtained from larvae of L2 *T. canis* cultured *in vitro* and the children who tested positive by this technique were evaluated by the technique of Western blot to determine the molecular weight of excretion-secretion proteins recognized by sera from patients with antibodies anti-*T. canis*. It was found that 12.5%. sera had antibodies against antigens of excretion-secretion for *T. canis*. Nine children were evaluated by the technique of Western blot and only 5 were positive for this technique, recognized two main antigens of 24 and 35 kDa. The analysis in sera from pediatric patients with epilepsy, showed a rate of 6.9% to antigens from *T. canis*, after analysis by ELISA and Western blot.

1683

MAPPING THE POTENTIAL RISK OF MYCETOMA IN SUDAN USING MAXIMUM ENTROPY ECOLOGICAL NICHE MODELLING

Abdallah M. Samy, A. Townsend Peterson

Kansas University, Lawrence, KS, United States

WHO recognized mycetoma as one of 17 neglected tropical diseases (NTDs) worldwide. Studies revealed a soil-borne mediated or thorn prick-mediated origin of mycetoma, but no studies are available to investigate the effect of soil type and Acacia distribution on mycetoma in Sudan. Here, we report efforts to investigate risk factors associated with mycetoma risk in Sudan using ecological niche modeling. Records of mycetoma cases were obtained from the scientific literature, PubMed, and GIDEON. Acacia records were obtained from the Global Biodiversity Information Facility. We developed ecological niche models (ENMs) based on digital GIS data layers summarizing soil, land-surface temperature, and greenness, summarizing environmental variation across Sudan. ENMs calibrated in endemic districts were transferred across all of Sudan, and suggested that greatest risk was in a belt across central and southern Sudan. We visualized mycetoma in environmental dimensions, and the results revealed that mycetoma in ecologically diverse landscapes under wide ranges of environmental conditions. We tested niche similarity between Acacia and mycetoma, and found significant niche similarity. These results revealed contributions of different environmental factors to mycetoma risk, identify suitable environments for disease emergence, raise the concerns for mycetoma-acacia association, and provide steps towards a robust, predictive risk map for the disease.

1684

THE EPIDEMIOLOGY OF ORAL HUMAN PAPILLOMAVIRUS INFECTION AMONG HEALTHY MEN AND WOMEN IN LIMA, PERU

Brian J. Rosen[1], Leora Leora Walter[2], Robert H. Gilman[1], Lilia Cabrerra[3], Patti E. Gravitt[1], Morgan A. Marks[1]

[1]Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, United States, [2]New York Hospital Queens, New York, NY, United States, [3]Asociacion Benefica Prisma, Lima, Peru

The incidence of head and neck cancers associated with human papillomavirus infection has been increasing in Peru. However, the burden of oral HPV infection in Peru has not been assessed in a healthy population. The objective of the study was to estimate the prevalence and correlates of oral HPV infection in healthy male and female residents from Las Pampas, a shantytown in Lima, Peru. A population-based sample of 1,500 healthy men and women between the ages of 8-85 in low to middle income areas of Lima, Peru was identified through random household sampling between January and August 2010. Adjusted odds Ratios (aOR with 95% CI) were used to assess the association of demographic factors, sexual practices, and oral hygiene on the prevalence of oral HPV infection. The prevalence of any HPV and any high-risk HPV (HR-HPV) was 6.8% and 2.0%, respectively. The three most common types were HPV 55 (3.4%), HPV 6 (1.46%), and HPV 16 (1.09%). Male sex (aOR, 2.32; 95% CI: 1.29, 4.18), age 19-27 (aOR, 2.77; 95% CI: 1.02, 7.56) and 46-55 years (aOR, 3.52; 95% CI: 1.07, 11.5) were significantly associated with prevalent HPV infection after adjustment. The prevalence of oral HPV in this population-based sample of healthy men and women from Peru was similar to estimates observed in the United States. Higher prevalence of oral infections in men were consistent with a male predominance of HPV-associated HNC and may signal a sex-specific etiology in the natural history of infection.

1685

DIAGNOSIS OF LIVER TUMORS USING IMAGE-BASED STATISTICAL FEATURES

Alyaa H. Ali, Intithar Malk, Sabah N. Mazhar

Department of Physics, College of Science for Women, Baghdad University, Baghdad, Iraq

Tumors located in the liver can be detected using CT imaging and comparison of intensity histograms with the associated statistical parameters for characterizing different regions of the liver including normal and cancerous. Identifying whether the tumor is benign or malignant is an important step in image-based liver cancer diagnosis. In this paper we describe an automated system for image-based liver segmentation of CT imagery using a multi-stage process. Each CT liver image is pre-processed to remove noise and enhance image quality to recognize structures within the liver. A key challenge is related to separating the liver from the rest of the abdominal cavity in CT imagery. We used a statistical feature descriptor to characterize healthy tissue versus cancerous regions and then applied a modified K-means classifier to improve the accuracy of the tumor segmentation process.

1686

ASSOCIATION OF HEMOGLOBIN LEVELS AND SELECTED BIOCHEMICAL MARKERS WITH DIABETIC NEPHROPATHY DISEASE

Khalid G. Al-Fartosi, Manal B. Al-Tamimi, Saad H. Al-Badry

Department of Biology, College of Science, University of Thi-Qar, Thi-Qar, Iraq

The present study was designed to investigate the relationship between hemoglobin levels (Hb), serum creatinine, lipid profile and urine albumin excretion in diabetic patients in Thi-Qar province, Iraq with various degrees

of nephropathy. The study cohort included 60 patients and 30 healthy subjects (control group) presented at the Al-Nasiriyah Endocrine Centre in Thi-Qar province, Iraq. The diabetic subjects were divided into three groups each with 20 subjects presenting diabetes mellitus (DM) disease for 1-5 years, 6-10 years and more than 10 years. The clinical results showed a significant decrease in the levels of Hb ($p<0.01$) in patients with DM compared to the control group. Also, there was a significant increase in blood sugar and urine albumin excretion in patients with DM compared with the control group ($p<0.01$). Serum creatinin increased significantly in patients with more than 10 years of DM compared with the control group. These results indicate a dyslipidemia in patients with DM compared with the control group.

1687

THE EFFECT OF METFORMIN ON GHRELIN SERUM LEVEL IN TYPE 2 DIABETES MELLITUS

Mohammed N. Mohammed, **Nahla O. Tawfik**

Department of Dental Basic Sciences, College of Dentistry, University of Mosul, Mosul, Iraq

Ghrelin is an orexigenic peptide hormone. A great deal of evidence suggests that ghrelin is involved in the development of Type 2 diabetes mellitus (DM). The aim of this study was to investigate the effect of metformin on ghrelin serum level in Type 2 DM patients. This clinical control study was carried out at the Al-Wafaa Medical Center for Diabetic and Endocrine Disorder patients in Mosul from October 2011 to March 2012. Fifty-five Type 2 diabetic patients and 20 control healthy subjects were enrolled. Patients and subjects were divided into 4 groups. Blood samples were collected from all subjects and the body mass index (BMI) was calculated for each person. Fasting blood sugar (FBS) level and ghrelin serum level were estimated for each patient. This study demonstrated a non-significant lower mean ghrelin serum level in the diabetic group compared to healthy controls. There were, however, significant differences in ghrelin serum levels between the diabetic group without metformin and the diabetic group treated with 1,000 mg metformin daily ($p < 0.05$). In this study we found that ghrelin serum levels had a negative correlation with age of patients over 30 years and BMI in both healthy and diabetic individuals.

1688

PEDIATRIC MENINGITIS IN THE AL-ABBASEYA FEVER HOSPITAL OF CAIRO

A. M. Al-Abbasi, Amro Shaee, Haifa I. Tawfeek

Infectious Diseases and Clinical Immunology, College of Medicine, Baghdad University, Baghdad, Iraq

Meningitis in children can be a fatal disease. Early discovery and prompt treatment using effective antimicrobials greatly reduces the mortality and resulting complications of the disease. Since meningitis is caused by a number of different microorganisms, detection of the causative agent is essential for proper treatment and to establish any preferential pattern of prevalence in different age groups or geographical regions. We conducted our clinical study on 61 patients admitted to Al-Abbasseya Fever Hospital in Cairo during the period of December 2010 to June 2011. The patients ranged in age from one month up to 17 years. Diagnosis of these cases was based on history, clinical data, laboratory tests, cerebrospinal fluid (CSF) examination and other visual diagnostic tests. There were 61 cases with 35 males and 26 females. Based on the CSF culture, gram stain, cell count and cell type diagnostic information, the patients were grouped into two classes: Acute bacterial meningitis (Group 1) and acute non-bacterial meningitis (Group 2). Group 1 consisted of 31 cases (20 males and 11 females) while Group 2 had 29 cases (15 males and 14 females), and one case of recurrent meningitis. The yield of microbial isolation from Group 1 was only 32.2% with four cases of N. meningitides, two cases of S. pneumonia, three cases of H. influenza and one case of Gram negative rods. The clinical and laboratory information and antimicrobial treatment regimen we used will be described. Pediatric meningitis needs special attention and a high rate of clinical suspicion as the yield of microbial isolation is low primarily due to the use of antibiotics prior to hospital admission. The choice of empirical antimicrobial usage might need to be reviewed from a clinical and public health perspective.

1689

UTILIZING THE COMMUNITY HEALTH WORKER NETWORK FOR LYMPHATIC FILARIASIS (LF) MORBIDITY MONITORING: THE DEVELOPMENT OF AN SMS-BASED SURVEILLANCE TOOL

Michelle C. Stanton[1], Square Z. Mkwanda[2], Alexander Y. Debrah[3], Linda B. Debrah[3], Achim Hoerauf[4], Nana-Kwadwo Biritwum[5], Abigail Best[1], Matthew Cliffe[1], Louise A. Kelly-Hope[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]Ministry of Health, Lilongwe, Malawi, [3]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [4]University of Bonn, Bonn, Germany, [5]Ghana Health Service, Accra, Ghana

Lymphatic filariasis (LF) is a parasitic infection that is responsible for over 15 million lymphoedema and 25 million hydrocele cases globally, resulting in LF morbidity being one of the leading causes of long-term disability. Currently, there are no standardised methods available for quantifying and mapping LF morbidity burden. The purpose of this study was therefore to pilot a novel method for collecting LF morbidity data in endemic areas of Malawi and Ghana. The premise of this method is that community health workers are able to quickly identify lymphoedema or hydrocele cases in their villages, but do not currently have a standardised method of collating this information. We have therefore developed an SMS-based tool which enables health workers to submit information on each identified case in their communities using a basic mobile phone. This tool was trialled under two scenarios: in March 2014 the tool was trialled by qualified, salaried health workers in southern Malawi; in May 2014 the study was repeated in Ghana using volunteer community health workers. In both scenarios, each health worker was asked to submit each identified case's village of residence, age, sex, condition and severity of condition (if lymphoedema) via SMS to a smartphone housed in-country. This information was then instantly compiled into a single database. A random sample of cases was visited by a medically qualified person to confirm the health workers' diagnoses, and GPS coordinates of their villages were recorded. The feasibility of the method was assessed in terms of the ease in which health workers were able to correctly identify cases (true positive rate), and the ease of use of the SMS-based tool (data entry error rates). A comparison between the performance of salaried health workers and volunteer health workers was also undertaken. Preliminary results for Malawi indicate that the true positive rate for reported lymphoedema and hydrocele cases using this method were 90% (95% CI [80%, 97%]), and 92% (95% CI [77%, 97%]) respectively.

1690

EPIDEMIOLOGY OF PODOCONIOSIS IN ETHIOPIA: RESULTS FROM A FIRST NATIONWIDE MAPPING

Kebede Deribe[1], Simon J. Brooker[2], Rachel L. Pullan[2], Heven Sime[3], Abeba Gebretsadik[3], Ashenafi Assefa[3], Amha Kebede[3], Asrat Hailu[4], Maria Rebollo Polo[5], Oumer Shafi[6], Moses J. Bockarie[5], Abraham Aseffa[7], Richard Reithinger[8], Fikre Enquselassie[9], Melanie J. Newport[10], Gail Davey[10]

[1]Brighton and Sussex Medical School and School of Public Health, Addis Ababa University, Addis Ababa, Ethiopia, Falmer, Brighton, United Kingdom, [2]Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]Ethiopian Public Health Institute, Addis Ababa, Ethiopia, [4]School of Medicine, Addis Ababa University, Addis Ababa, Ethiopia, [5]Center for Neglected Tropical Diseases, Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [6]Federal Ministry of Health, Addis Ababa, Ethiopia, [7]Armauer Hansen Research Institute/ALERT, Addis Ababa, Ethiopia, [8]International Development Group, Research Triangle Institute, Washington, DC, United States, [9]School of Public Health, Addis Ababa University, Addis Ababa, Ethiopia, [10]Brighton and Sussex Medical School, Falmer, Brighton, United Kingdom

Podoconiosis (endemic non-filarial elephantiasis) is a major cause of tropical lymphoedema and is endemic in Ethiopia. To guide targeting and implementation of the National Neglected Topical Diseases control strategy, an integrated nationwide survey of lymphatic filariasis (LF) and podoconiosis was conducted between June and September 2013. Here we present a description of podoconiosis epidemiology in Ethiopia resulting from this survey. District health offices' reports of podoconiosis and LF were used to guide selection of survey sites. Data and cluster level GPS coordinates were collected via smartphones by trained local health workers. Individual level data were available for 129,959 randomly-sampled individuals from 1,315 communities in 659 districts. Blood samples were tested for *Wuchereria bancrofti* antigen using immunochromatographic card tests (ICT). A clinical algorithm was used to diagnose podoconiosis by excluding other potential causes of lymphoedema of the lower limb. Mixed-effects logistic regression was used to identify individual-level correlates, adjusting for dependence within district and municipality. Overall, 8,110 of 129,959 (6.2%, 95%CI; 6.1 to 6.4%) surveyed individuals were identified with lymphoedema with 5253 (4.0%, 95% CI; 3.9 to 4.1%) confirmed as podoconiosis cases. Prevalence among men and women was 3.4% (95%CI; 3.3 to 3.5%) and 4.7% (95%CI; 4.5 to 4.8%) ($p<0.001$), respectively. During the survey 85.2% (95%CI: 84.9 to 85.3%) of respondents were wearing shoes, but only 57.9% (95%CI: 57.6 to 58.2%) of them were wearing protective shoes. Female sex, older age, wearing shoes after 12 years of age, washing feet less frequently than daily were significantly associated with increased odds of having podoconiosis. Attending formal education, living in a house with a covered floor were associated with decreased odds of having podoconiosis. The survey confirmed that podoconiosis remains a significant public health problem and is widely distributed in Ethiopia; it is endemic throughout 30% of the country's landmass, where more than 40% of the population live. Results provide a current benchmark of the burden of the disease, against which future podoconiosis control programmes can be measured.

1691

EFFECTS OF ELIMINATION CAMPAIGN OF LYMPHATIC FILARIASIS SEEN IN NEPAL

Harishchandra Shah

Ministry of Health and Population, Eastern Health Development Region,Dhankuta, Nepal, Dhankuta, Nepal

Effects of Elimination Campaign of lymphatic Filariasis seen in Nepal, 2014* Lymphatic filariasis (LF) is one of the five infectious diseases targeted by WHO for elimination as public health problem in Nepal. They are LF, Kala-azar, Leprosy, Yaws and Chagas disease. WHO launched GPELF with a goal to eliminate LF as public health problem by 2020 A.D. Two key strategies are: Interruption of transmission of LF infection in endemic countries by reducing microfilariae prevalence levels (below 1%) through Mass Drug Administration (MDA); Prevention and alleviation of disabilities and sufferings in individuals already affected by LF. Nepal: A total of 61 districts are considered LF endemic. Some districts with high prevalence are as high as 40% . The population at risk in Nepal are 25 millions. The causative agent are *Wuchereria bancrofti*, and transmission vector is Culex quinquefasciatus. The reported chronic conditions in 2012 are 28,835, majority were hydrocele. The 10 most morbid districts with hydrocele were Morang, Jhapa, Bardia, Banke, Saralahi, Dhading, Nuwakot,Kapilbastu, Bara, and Mahottari.LF Elimination Strategies: Interruption of transmission by Mass Drug Administration (MDA) using two drugs regimen, Diethylcarbamazine (DEC) and Albendazole, once yearly for six years. Morbidity management by self care and with support using intensive but simple, effective and local hygiene technique.MDA 2013: The number of MDA districts were 56. The total population in MDA districts was 25087450. The estimated eligible population for MDA: Phase I : 37 districts of eastern, central and western regions and Phase II : 19 districts of mid western and far western regions.

1692

REAL-TIME PCR AND MELT-CURVE ANALYSIS (QPCR-MCA) AS A REFERENCE LABORATORY TOOL FOR THE DETECTION OF *ONCHOCERCA VOLVULUS* AND ITS IMPORTANCE FOR MONITORING AND EVALUATION ACTIVITIES

Elizabeth A. Thiele[1], Vitaliano A. Cama[1], Thomson Lakwo[2], Sindew Mekasha[3], Paul T. Cantey[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Ministry of Health, Kampala, Uganda, [3]Ethiopian Public Health Institute, Addis Ababa, Ethiopia

The efforts to eliminate onchocerciasis in most of Africa by 2025 necessitate re-examination of current monitoring and evaluation tools. In particular, assessment of hypoendemic zones, stop-treatment determinations, and post-intervention surveillance will require sufficiently sensitive tools to detect low-intensity infections. Mass drug administration with ivermectin (IVM) is expected to decrease microfiladermia and thereby decrease the usefulness of skin snip microscopy, currently the standard assessment tool. Using a pan-filarial qPCR-MCA, we assessed 1) the utility of this single-step assay for detecting evidence of microfilaria (MF) in residual skin snips and 2) the sensitivity of skin snip microscopy relative to our PCR-based assay. Specificity of the qPCR-MCA to *Onchocerca volvulus* was verified using DNA from *O. volvulus* macrofilariae; MF of *B. malayi* and *pahangi*, *L. loa*, *M. ozzardi*, and *W. bancrofti*; and uninfected human controls. Utility of the qPCR-MCA assay and the relative sensitivity of microscopy were evaluated with residual skin biopsies (i.e., after 24-hour incubation in saline) collected from hyperendemic regions of Uganda and Ethiopia (n=500 each) which had received limited rounds of IVM. qPCR-MCA detected over 94% of known positive skin snips (139/147 total microscopy positive), identifiable by consistent, well-defined dissociation curves at 79.35°C (S.D. 0.22) with a minimum 1°C difference from other filarial species. Using qPCR-MCA as the reference test, the sensitivity of the skin snip microscopy was only 74.7% (121/162) and 28.1% (18/64) in Uganda and Ethiopia, respectively. Combined across countries, qPCR-MCA detected an additional 87 positive samples (38.5%), indicating a combined microscopy sensitivity of 61.5% (139/226). When evaluating low-intensity infections (≤2 MF/snip), the sensitivity of microscopy was only 46% (74/154). Thus, skin snip microscopy does not appear to be sufficiently sensitive to assess transmission in areas with low microfiladermia or to make stop-treatment decisions in the absence of other transmission assessments (e.g., vector data). qPCR-MCA can augment sensitivity and provide diagnostic confirmation of skin biopsies and will be useful for validating new monitoring tools that may be developed to support elimination efforts.

1693

FIFTY-EIGHT YEARS OF MAN AGAINST THE WORM IN BUDONGO ONCHOCERCIASIS FOCUS OF UGANDA-INTERRUPTION OF TRANSMISSION IS FINALLY IN SIGHT

Moses N. Katabarwa[1], Tom Lakwo[2], Peace Habomugisha[3], Ephraim Tukesiga[4], Edson Byamukama[3], Edson Byamukama[3], Galex Orukan Ochieng[5], William Mugayo[6], Thomson Isingoma[6], Moris Okumu[7], Martini Mborekere[8], David Oguttu[9], Annet Khainza[3], Edridah Tukahebwa[9], Rolf Garms[10], Tom Unnasch[11], Frank O. Richards[12]

[1]The Carter Center/Emory University, Atlanta, GA, United States, [2]Ministry of Health, Vector Contol Division, Kampala, Uganda, [3]The Carter Center, Kampala, Uganda, [4]Kabarole District Health Services, FortPortal, Uganda, [5]Mbale District Health Services, Mbale, Uganda, [6]Masindi Health Services, Masind, Masindi, Uganda, [7]Bulisa District Health Services, Bulisa, Uganda, [8]Hoima District Health Services, Hoima, Uganda, [9]Ministry of Health, Vector Control Division, Kampala, Uganda, [10]Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany, [11]Department of Global Health, University of South Florida, Tampa, FL, United States, [12]The Carter Center, Atlanta, GA, United States

The battle against onchocerciasis in hyperendemic Budongo focus (where the baseline microfilaria (mf) rate was 80% and fly infection rate 20%) has lasted over a half a century. Vector control of *Simulium neavei* using DDT commenced in1955, and larviciding was halted when no larval/pupal stages of *S. neavei* were observed on crabs, and no adults flies were found in human landing captures. Six months after stopping larviciding, however, rapid repopulation of *S. neavei* was observed. In August 1956, a new series of DDT larviciding was initiated. In 1958, the early stages of the vector could not be found in the rivers and the adult fly had disappeared by 1962 indicating elimination of *S. neavei* from the focus. Repeated courses of DEC were provided to individuals in the communities who were infected. To avoid vector reinfestation, maintenance larviciding continued until about 1971. Political unrest in Uganda led to the collapse of this work, and by 1989 *S. neavei* had again repopulated the area and onchocerciasis recrudescence had occurred. Annual mass drug administration (MDA) with ivermectin was provided from 1989 to 2007 to all the 184 affected communities and a population of 150,195 people. However, a 2008 serosurvey of 3159 children showed an OV16 antibody rate of 9.5%, indicating continued transmission. After Uganda established a policy for onchocerciasis elimination in 2007, biannual treatment was launched in Budongo in 2008, and continues to date. However, in assessments done in 2011 vector infectivity rates still ranged up to 8.7%. In June 2012, temephos (Abate®) larviciding was added to compliment twice per year ivermectin treatments. By February, 2014, only 2 (0.7%) crabs out of 300 were infested; no adult fly has been collected since September, 2013. Budongo focus is an example of a difficult onchocerciasis 'hot spot' requiring both twice yearly ivermectin MDA and vector control to break transmission.

1694

EVALUATION OF LYMPHATIC FILARIASIS AND ONCHOCERCIASIS IN THREE SENEGALESE DISTRICTS TREATED FOR ONCHOCERCIASIS WITH IVERMECTIN FOR MORE THAN 15 YEARS

Nana O. Wilson[1], Alioune Badara Ly[2], Ngayo Sy[3], Lamine Diawara[4], Abdel Direny[5], Mawo Fall[6], Vitaliano A. Cama[1], Paul T. Cantey[1], Mark L. Eberhard[1], LeAnne M. Fox[1], Daouda Ndiaye[7], Christine Dubray[1]

[1]Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Ministère de la Santé et de l'Action Sociale, Dakar, Senegal, [3]Service de Lutte Antiparasitaire, MSAS, Dakar, Senegal, [4]World Health Organization and African Programme for Onchocerciasis Control Representative, Dakar, Senegal, [5]IMA World Health, Washington, DC, United States, [6]RTI/ENVISION, Dakar, Senegal, [7]Cheikh Anta Diop University, Dakar, Senegal

In Africa, there is significant overlap between onchocerciasis and lymphatic filariasis (LF). Current efforts to eliminate these two diseases are through mass drug administration (MDA) with ivermectin alone for onchocerciasis or with ivermectin and albendazole for LF. Years of ivermectin distribution for onchocerciasis may have decreased or interrupted LF transmission in certain areas. The Kedougou region, Senegal, has been historically known to be co-endemic with LF (immunochromatographic test [ICT] prevalence ≥1%) and onchocerciasis (microfilaria prevalence 9.0-68.4%). MDA for onchocerciasis started in 1988 and as of 2014, albendazole had not been added to target LF. The objective was to assess in an integrated manner the status of LF and onchocerciasis in three districts of Kedougou after ≥15 years of ivermectin MDA. Sixteen villages close to rivers and breeding sites for onchocerciasis vector, *Simulium spp,* were selected. LF antigenemia testing (ICT) was added to skin snip microscopy for onchocerciasis evaluation. Convenience sampling of residents ≥5 years was performed. Dried blood spots were collected to test for antibodies against Wb123 (LF) and Ov16 (onchocerciasis) antigens (results pending). One village refused to participate and one was excluded because it was treated with ivermectin too recently. Forty percent (1154/2925) of residents participated; 50% were males and the median age was 15 years. In two districts, no participants were ICT or skin snip positive. In the third district, 3.4% (6/176) were ICT-positive (village range 1.9-6.4%) and 0.7% (1/150) were skin snip-positive. The mean age of ICT-positive participants was 49 years (range 25-79); the participant with a positive skin snip was 79 years old. After ≥15 years of ivermectin distribution, LF prevalence was still above treatment threshold in one of the three districts included in the evaluation. The integrated evaluation of LF and onchocerciasis provided important information on both diseases that should help program managers make decisions about treatment interventions.

1695

THE USE OF HUMAN SWEAT METABOLITES AS BAIT FOR MONITORING VECTORS OF ONCHOCERCIASIS IN WEST AFRICA AND LATIN AMERICA

Ryan M. Young[1], Nathan D. Burkett-Cadena[1], Tommy W. McGaha[2], Mario A. Rodríguez-Pérez[3], Laurent Toé[4], Bill J. Baker[1], Raymond Noblet[2], Eddie W. Cupp[5], Andrew J. Shilling[1], Javier Garza-Hernández[3], Kristel Rodríguez-Morales[3], José Torres-Estrada[6], Thomas R. Unnasch[1]

[1]University of South Florida, Tampa, FL, United States, [2]University of Georgia, Athens, GA, United States, [3]Instituto Politécnico Nacional, Reynosa, Mexico, [4]Multidisease Surveillance Centre, World Health Organization, Ouagadougou, Burkina Faso, [5]Auburn University, Auburn, AL, United States, [6]Instituto Nacional de Salud Pública, Tapachula, Mexico

Onchocerciasis, a.k.a. river blindness, is a parasitic disease caused by infection from the nematode *Onchocerca volvulus*. The parasite is transmitted to humans by the bite of infected black flies (genus

Simulium). The World Health Organization (WHO) estimates 18 million people suffer from onchocerciasis, the vast majority occurring in central Africa, and isolated foci in the American Tropics. Treatment of the disease relies on accurate epidemiological data, which is best achieved though real-time data of infection prevalence in the vectors. The need for a new monitoring method is crucial. To this end we identified key primary metabolites in human sweat, which putatively attract black flies to humans. Laboratory studies were then conducted in Southern Mexico and West Africa to test which compounds attracted these vectors of onchocerciasis, using electroantennography and y-tube olfactometry. The attractive compounds will be developed into baits to lure black flies to a novel trap for monitoring vector abundance and infection prevalence in both Latin America and Africa. In this study we describe the identification of key human sweat components via GC-MS, the identification of attractive metabolites to the two major species of *Simulium* as well as trap development and bait formulation for the continued monitoring of these important disease vector.

1696

LYMPHATIC FILARIASIS ELIMINATION: ASSESSMENT OF TWO VILLAGES WITH DIFFERENT ENDEMICITY LEVELS IN A PREVIOUSLY HIGHLY ENDEMIC REGION (SIKASSO) OF MALI

Yaya Ibrahim Coulibaly[1], Salif Seriba Doumbia[1], Ilo Dicko[1], Lamine Soumaoro[1], Massitan Dembele[2], Sekou Fantamady Traore[1], Joseph Kubofick[3], Amy Klion[3], Louise Kelly-Hope[4], Thomas B. Nutman[3], Moses J. Bockarie[4]

[1]ICER Mali, Bamako, Mali, [2]National Lymphatic Filariasis Elimination Program, Mali, Bamako, Mali, [3]National Institutes of Health, Bethesda, MD, United States, [4]CNTD, Liverpool School of Tropical Medicine, United Kingdom, United Kingdom

Lymphatic filariasis (LF) is a disfiguring neglected tropical disease that is targeted for elimination by 2020 using annual mass drug administration (MDA) in endemic areas. We previously reported the *Anopheles gambiae* complex frequencies in 2 neighboring villages of Kolondièba, one of the highly LF endemic district of Sikasso, Mali. To assess transmission interruption after 6 annual MDA in the district of Kolondièba and determine the potential impact of vector density on MDA-induced transmission interruption. A cross sectional study with quantitative and qualitative data collection methods in 2 villages of Sikasso region. The village of Boundioba had a lower *Anopheles* density as compared to Bougoula (1,494 versus 251 specimen from July to December 2011). A total of 481 volunteers in Bougoula including 340 female (70.5%) and 332 in Boundioba including 221 female (66.6%) were included in this study. The 6-7 years/15 years and above composition was 113/368 and 127/205 respectively in Bougoula and Boundioba**.** Microfilaremia was significantly more frequent in the15 years and above in Boundioba(1.95%, 4/205) as compared toBougoula (0%, 0/368), (p=0.02, Fisher exact test). Additionally, the 2 villages showed comparable low prevalences in 6-7 years olds with respectively 1/127 and 0/113 for Boundioba and Bougoula. *Anopheles* vector density may be misleading because it is not necessarily associated with a higher endemicity in a village under MDA.

1697

POTENTIAL RE-EMERGENCE OF *WUCHERERIA BANCROFTI* TRANSMISSION IN A PREVIOUSLY CONTROLLED HYPERENDEMIC REGION (SIKASSO) IN SOUTHERN MALI

Siaka Yamoussa Coulibaly[1], Yaya Ibrahim Coulibaly[1], Ilo Dicko[1], Siaka Konate[1], Housseini Dolo[1], Moussa Brema Sangare[1], Michel Emmanuel Coulibaly[1], Lamine Soumaoro[1], Abdallah Amadou Diallo[1], Modibo Sangare[1], Benoit Dembele[1], Massitan Dembele[2], Pj Hooper[3], Eric Ottesen[3], Dominique Kyelem[3], Sekou F. Traore[1], Amy D. Klion[4], Thomas B. Nutman[4]

[1]ICER Mali, Bamako, Mali, [2]National Lymphatic Filariasis Elimination Program, Mali, Bamako, Mali, [3]Neglected Tropical Diseases Support Center, Task Force for Global Health, Atlanta, GA, United States, [4]National Institute of Allergy and Infectious Diseases/National Institutes of Health, Bethesda, MD, United States

Mass drug administration (MDA) for the elimination of lymphatic filariasis (LF) has led to potential transmission interruption in several endemic regions of Mali based on transmission assessment surveys (TAS). To assess the utility of TAS approach, we utilized standard TAS methodology (ICT positive prevalence in 6-7 year olds) and compared it to xenomonitoring, night blood microfilariae counts and IgG4 antibody to Wb123 (for the last 3 years) over a five year period (2009-2014) following the cessation of MDA in 6 villages in the region of Sikasso in southern Mali. In 2009 (at the start of the surveillance period) all 289 children aged 6-7 years were negative for circulating filarial antigen (CFA) by ICT, by calibrated thick smears of blood collected at night, and by IgG4 antibody to Wb123. Despite this, 2/4391 (0.11%) dissected mosquitoes were positive for larvae of *Wuchereria bancrofti* (Wb). In 2011, there was a CFA prevalence by ICT of 2.6% (8/301) in the 6-7 year olds, a prevalence of 1.09% (1/92) for antibody responses to Wb123, but negative xenomonitoring. In the subsequent 2 years (2012 and 2013), there were consistent and significant increases in the prevalence of CFA (Trend Chi^2= 11.49, p=0.0007) to 3.9% (11/285) in 2012, and 4.1% (13/316) and in the prevalence of anti-Wb123 IgG4 to 3.2% (10/316) in 2013. Despite this increase in both ICT and Wb123 IgG4 antibody prevalence, no infected anopheline mosquitoes were found in 2011, 2012 and 2013. These data suggest that despite having met the criteria for cessation at the beginning of the surveillance, that there appears to be low level emergence of Wb transmission and that antibody monitoring may provide a better early warning tool than more standard TAS tools.

1698

SYSTEMIC NON-COMPLIANCE: A POTENTIAL FACTOR INTHE RE-EMERGENCE OF LYMPHATIC FILARIASIS TRANSMISSION IN SIKASSO, MALI

Ilo Dicko[1], Yaya Ibrahim Coulibaly[1], Siaka Yamoussa Coulibaly[1], Housseini Dolo[1], Moussa Brema Sangare[1], Siaka Konate[1], Michel Emmanuel Coulibaly[1], Lamine Soumaoro[1], Abdallah Amadou Diallo[1], Modibo Sangare[1], Benoit Dembele[1], Massitan Dembele[2], Pj Hooper[3], Eric Ottesen[3], Dominique Kyelem[3], Thomas B. Nutman[4], Sekou Fantamady Traore[1], Amy D. Klion[4]

[1]ICER Mali, Bamako, Mali, [2]National Lymphatic Filariasis Elimination Program, Mali, Bamako, Mali, [3]Neglected Tropical Diseases Support Center, Task Force for Global Health, Atlanta, GA, United States, [4]National Institute of Allergy and Infectious Diseases/National Institutes of Health, Bethesda, MD, United States

Recent transmission assessment surveys (TAS) suggest low level re-emergence of *Wuchereria bancrofti* transmission after cessation of mass drug administration (MDA) in 6 previously hyperendemic villages in Sikasso, Mali. Coverage rates in the villages ranged from 67% to 89.6% over the 7 years of MDA and all stopping criteria were met at the beginning of the surveillance period. To begin to identify potential causes for this re-emergence, a questionnaire was administered to randomly selected adult residents of the six villages to assess the prevalence of and

reasons for systematic non-compliance with MDA. A total of 486 subjects (170 men and 316 women) were questioned, of whom 16.1% (79/486) reported never swallowing MDA drugs. The most common reasons given were being unaware of MDA (24/486; 4.9%), being pregnant or breast-feeding (8/486; 1.6%) and not willing to take the drugs (6/486; 1.2%). Although systematic non-compliers were more likely to be younger [OR = 1.7 (1.006-2.921) for individuals 15-30 vs. >30 years of age], compliant and systematically non-compliant subjects were similar with respect to participants' instruction level [OR = 1.2 (0.59-2.51)] and the presence of lymphoedema / hydrocele [OR = 0.5 (0.11-2.63)]. These data suggest that significant rates of systematic non-compliance can be present despite adequate overall coverage rates. Whether persistent infection in systematic non-compliers provided the reservoir for re-emergence of transmission in the 6 study villages requires further study.

1699

NON-*MANSONELLA OZZARDI* ATYPICAL MICROFILARIASIS IN THE PERUVIAN AMAZON BASIN

Salomon Durand[1], Dolores Rimarachin[2], Amy C. Morrison[2], Karen Campos[2], Jorge Nunez[1], Moises Sihuincha[3], Geral C. Baldeviano[1], Andres G. Lescano[1]

[1]U.S. Naval Medical Research Unit - 6, Callao, Peru, [2]U.S. Naval Medical Research Unit - 6, Iquitos, Peru, [3]Hospital Apoyo Iquitos, Iquitos, Peru

Filariasis is a neglected tropical disease around the world. This disease is a vector-borne infection caused by nematodes (roundworms) of the family Onchocercidae. Since 1950s, filariasis caused by *Mansonella ozzardi* has been regularly reported in the Peruvian Amazon basin. Isolated cases have been diagnosed as *M. perstans*, *Bruguia* spp, *Onchocerca* spp. and *Dirofilaria* spp. Since 2010, by examining de-identified blood samples (thick smear and Knott's concentration techniques) we have characterized the prevalence of filarial infections and associated clinical symptoms in both rural (n=383) and urban (n=755) human populations located on tributaries of the Amazon River near and in Iquitos, Peru. In the rural communities, prevalence of microfilariae was 28.5% (109/383) overall; but the majority 99% (108/109) were *M. ozzardi.* Prevalence rates were not heterogeneous ranging from 0 to 72.5% in 11 communities. In contrast, of 755 samples from residents of Iquitos, 2% and 4% were infested with *M. ozzardi* and an atypical microfilaria, respectively. Interestingly, those infected with *M. ozzardi* tended to be febrile adult males with occupations associated with rural areas, whereas those infested with the atypical parasite were rarely febrile and were often children or housewives. Surveillance in local hospitals identified at least one morphologically distinct atypical microfilariae and one co-infection with *M. ozzardi* in seven symptomatic patients; one had subacute skin lesions and the others fever. The atypical microfilariae were macroscopically distinct from *M. ozzardi*, measuring 600 x 8 µm, with no sheath and no nuclei in the tail. In a subset of samples tested by PCR. All *M. ozzardi* were confirmed, but five of the atypical microfilariae tested negative the internal transcribed spacer rDNA sequence of *M. perstans* and *Onchocerca volvulus.* In conclusion, atypical microfilariae with a distinct epidemiology from *M. ozzardi*, and not related to *M. perstans,* or *O. volvulus*, are sufficiently prevalent to warrant investigation of their health impact in the Peruvian Amazon.

1700

INTEGRATED FILARIAL MICRO-MAPPING TO DETERMINE IMPLEMENTATION STRATEGIES IN *LOA LOA* CO-ENDEMIC AREAS: THE ANGOLAN EXPERIENCE

Miguel Brito[1], Rossely Pauloi[1], Tom Unnasch[2], Robert Novak[2], Benjamin Jacob[2], Michelle Stanton[3], David Molyneux[3], Louise Kelly-Hope[3]

[1]CISA - Health Research Centre in Angola, Caxito, Angola, [2]College of Public Health, Department of Global Health, University of South Florida, Tampa, FL, United States, [3]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

The co-distribution of *Loa loa* (loiasis or tropical eye worm) is a significant impediment to the lymphatic filariasis (LF) and onchocerciasis elimination programmes in Angola, due to the potential risk of severe adverse events (SAEs) associated with the drug ivermectin when given to individuals with high *L. loa* microfilarial loads in the blood. This has significant implications for mass drug administration campaigns and alternative strategies may be required in selected areas. One of the highest *L. loa* risk areas is Dande Municipality, Bengo Province in Northern Angola, an area which historically has reported cases of LF and onchocerciasis. To better determine the safest treatment strategy in this area, this study conducted an integrated filarial micro-mapping survey to understand the geographical overlap of the three diseases. GIS-Remote Rapid Eye satellite data was also employed to provide the foundation for empirical information on vector and parasite populations. In total 23 villages, distanced approximately 10-15km apart, across peri-urban and rural areas were surveyed during January-February 2014. In each village, up to 100 individuals were assessed using the rapid assessment procedure for loiasis (RAPLOA) and rapid epidemiological mapping of onchocerciasis (REMO), and two questions on LF morbidity (presence of lymphedema, hydrocele). The study found low levels of endemicity of all three diseases (<20%), with different overlapping distributions, with most villages reporting at least one filariasis case. To confirm the hypo-endemic levels of LF and onchocerciasis, a further seroprevalence survey using rapid diagnostic tests in the same villages is planned for June 2014. This will provide additional micro-epidemiological information to help determine if the recommended alternative strategy of albendazole twice yearly and long-lasting/insecticide treated bednets (LLINs/ITNs) should be used for LF elimination, and if an alternative to ivermectin for hypoendemic onchocerciasis elimination, such as the drug doxycycline or vector control, needs to be considered.

1701

SERO-PREVALENCE AND RISK FACTOR SURVEY FOR LYMPHATIC FILARIASIS IN PAPUA NEW GUINEA

Melinda Susapu[1], Moses Bockarie[2], Lisa Reimer[2], Louise Kelly-Hope[2]

[1]Department of Health, Port Moresby, Papua New Guinea, [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Papua New Guinea (PNG) has an estimated population of 7 million inhabitants; of which 4 million are predicted to be at risk of lymphatic filariasis (LF). LF is a debilitating disease caused by lymphatic-dwelling nematodes *Wuchereria bancrofti*, which is transmitted by *Anopheles* mosquitoes in PNG, similar to malaria. Some national and published data exists, however, the geographical distribution, burden of disease and associated risk factors are currently not well defined. This study investigated the prevalence and potential risk factors of LF in one endemic province of PNG through the administration of a prevalence survey and household questionnaire related to the LF transmission and the national LF programme. In April 2013, four villages in a southern remote area of Madang Province were selected. Approximately 100 individuals in each village were interviewed and examined for LF infection, using ICT rapid diagnostic kits, and evidence of clinical disease. This study found that 32 individuals out of 389 surveyed (8.2%) were LF antigen positive, and 3 individuals had lymphodema (elephantiasis) of the leg (0.8%). All of those

interviewed did not know about the disease, what caused it, how it was transmitted or were aware of the national programme to eliminate LF. A follow-up microfilaremia (MF) survey was conducted in the study site with the most ICT positive individuals, and included the majority of community members (n=300). Preliminary results indicate the average Mf prevalence was 41.5%, and ranged from 10.5% in children under 10 years, to 54.6% in adults over 50 years of age. Mf prevalence was found to be higher in males (46.4%) than females (34.9%), and among those living in houses made of bush material (45.8%) compared with other semi-permanent materials (23.1%). The field work is still in progress and expected to be finish with final results by November 2014. This research highlights that LF is endemic in remote areas of the country and the national LF programme has to scale up its efforts to control and eliminate the spread of infection with particular emphasis on LF advocacy and education to those most at risk.

1702

METHODS FOR ASSESSING LYMPHATIC FILARIASIS TRANSMISSION IN LOW ENDEMIC AREAS OF BANGLADESH: ONE STEP CLOSER TO THE ELIMINATION GOAL

A. S. M. Sultan Mahmood[1], Rouseli Haq[1], Mujibur Rahman[1], Shamsur Rahman[1], Louise Kelly-Hope[2]

[1]Directorate General of Health Services, Dhaka, Bangladesh, [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Bangladesh had one of the highest burdens of lymphatic filariasis (LF) at the beginning of the Global Programme to Eliminate LF (GPELF), with an estimated 80 million people at risk of infection. Baseline mapping in 2000 using the rapid diagnostic ICT cards, found that 34 of the 64 districts in the country were endemic, however, only 19 districts required mass drug administration (MDA) using the drugs albendazole and DEC to interrupt transmission. The Bangladesh LF Programme has successfully scaled up MDA in these districts, and is moving into the elimination phase and using the WHO recommended Transmission Assessment Survey (TAS) to assess their success in interrupting transmission - so far with excellent results. The outstanding important question for the LF Programme was how to assess the 15 endemic districts that were found to have low prevalence levels (<1%) and not be eligible for MDA. Follow-up night blood microfilaria (Mf) and community clinical surveys undertaken in 2008 -2010 in selected areas of these districts found little or no evidence of infection and disease such as lymphedema and hydrocele. Currently, there is no recommended strategy for assessing low endemic districts, therefore, in order to address this issue and provide more rigorous evidence that LF is not a public health problem, the TAS method is being used as an assessment tool with additional systematic patient searching at household level. The assessments are planned for each month of 2014 and being carried out by trained field teams visiting schools for TAS (targeting children) and using local community clinic workers and volunteers to visit households (targeting individuals with clinical manifestations). To date five districts have been assessed with good results, and if the remaining districts are also found to have little or no LF infection or disease, the national LF programmes can 'shrink the LF map' by approximately 30 million people and move one step closer to their elimination goal, with an increased focus on the new priorities of surveillance and morbidity management.

1703

SYSTEMATIC REVIEW AND META-ANALYSIS OF DOXYCYCLINE IN CONTROL OF LYMPHATIC FILARIASIS

Patricia A. Woodall

Emory University, Atlanta, GA, United States

Lymphatic Filariasis control programs rely on periodic population-wide administration of microfilaricidal agents repeated over several years to decrease transmission. Effective macrofilaricidal agents could decrease the years necessary to achieve elimination. Doxycycline has emerged as a possible agent due to its effects on the Wolbachia endosymbiont. A systematic review identified 9 published randomized controlled trials evaluating the effects of various Doxycycline regimens on *Wuchereria* or *Brugia* microfilaria levels at 12 months post-treatment in 5 countries, with or without interim single-dose microfilaricide (Ivermectin or DEC). For elimination of microfilariae at 12 months (compared to placebo), the pooled Risk Ratio was 3.22 (1.95, 5.32) , with high heterogeneity (I^2=68%). Subgroup analysis showed: Doxycycline 6-8 week regimens, RR= 3.96 (2.07, 7.59); Doxycycline 3-4 week regimens, RR= 2.14 (1.08, 4.26); Ivermectin or DEC 4 months after Doxycycline, RR= 2.29 (1.73, 3.04); no interim microfilaricide, RR= 5.80 (2.36, 14.24). Multi-day Doxycycline regimens effectively eliminate LF microfilariae at 1 year after treatment. Applicability of such multi-day regimens to population-wide control programs is limited. Further studies should evaluate shorter-term treatment.

1704

SIMULTANEOUS DETECTION OF *ONCHOCERCA VOLVULUS* AND *O. OCHENGI* IN INFECTED *SIMULIUM* FLIES USING ANEW MULTIPLEX REAL-TIME PCR

Anna Albers[1], Christina Weier[1], Sandra Arriens[1], Samuel Wanji[2], **Kenneth Pfarr**[1], Achim Hoerauf[1]

[1]University Hospital of Bonn, Bonn, Germany, [2]Research Foundation for Tropical Diseases and Environment, Buea, Cameroon

Onchocerca volvulus parasitic worms infect ~37 million people in sub-Saharan Africa and parts of Latin America, causing dermatitis, skin atrophy and visual impairment. In 27 endemic countries in Africa, 130 million people at risk of the disease. The January, 2014 report of the International Task Force for Disease Eradication has stated the continued need for improved diagnostics to assess when mass drug administration efforts can be halted and to monitor for recrudescence. Currently transmission is monitored by identifying larvae in dissected *Simulium damnosum* species, the vector of *O. volvulus*, which are also able to transmit *O. ochengi*, a parasitic worm of cattle that does not infect humans. We developed a multiplex real-time PCR based on the *ND5* gene of the Onchocercidae genus with specific TaqMan probes to differentiate *O. volvulus* and *O. ochengi* from other Onchocercidae. A blinded study with 217 flies from *O. volvulus* and *O. ochengi* endemic and *O. volvulus*/*O. ochengi* co-endemic areas in Cameroon (n=23) showed 100% specificity in all analyzed *Simulium* flies. Vector monitoring to assess transmission potential in endemic areas is reliable. Our multi-plex real-time PCR offers time and cost savings over species identification via microscopy.

1705

MODELING THE EFFECTS OF MASS DRUG TREATMENTS AND VECTOR CONTROL ON CO-INFECTION WITH MALARIA AND LYMPHATIC FILARIASIS

Sarah Lukens, Brajendra K. Singh, Edwin Michael

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

Malaria and lymphatic filariasis (LF) are both transmitted by anopheles mosquitoes and are co-endemic in many regions of the tropics. For ongoing global campaigns to eliminate LF and malaria, it is important to understand interactions between both parasites and hosts where they co-exist. The use of mass drug administrations (MDA) to reduce prevalence and intensity of microfilariae may increase the lifespan of anopheles mosquitoes and thereby potentially increasing the transmission of malaria, while interactions at the host level may affect susceptibility, disease severity, and co-transmission of both diseases. Each parasite system alone exhibits complex dynamics where factors such as vector biting rates and threshold prevalence of human infection contributes to either extinction or stabilization to an endemic level. Knowledge of how interactions between both systems may affect co-infection endemicity and extinction dynamics is important for designing effective disease management programs such as MDA and vector control (VC). We extend a mathematical model of

malaria-LF co-infection to describe how the interplay between these two infections influence threshold behaviors in the system, and how MDA and VC interventions can influence elimination or resurgence of both diseases.

1706

IMPACT OF MEROWE DAM ON ONCHOCERCIASIS VECTORS OF ABU HAMED, NORTHERN SUDAN

Isam Zarroug[1], Arwa Elaagib[2], Sara Abuelmaali[3], Hanan Mohamed[1], Wigdan Elmubarak[1], Kamal Hashim[4], Tong Chor M Deran[1], Nabil Aziz[5], Tarig Higazi[6]

[1]National Onchocerciasis Control Program, Khartoum, Sudan, [2]University of Khartoum, Khartoum, Sudan, [3]National Health Laboratories, Khartoum, Sudan, [4]National Programme for Prevention of Blindness, Khartoum, Sudan, [5]The Carter Center, Khartoum, Sudan, [6]Ohio University, Zanesville, OH, United States

Abu Hamed, the northernmost onchocerciasis focus in the world, is located along the River Nile banks in the Nubian Desert. Hydroelectric dams can alter activity of black flies and may provide breeding sites for black fly. Merowe Dam, the largest hydropower project in Africa, was built west of Abu Hamed focus in 2009. The impact of the Dam on onchocerciasis and its black fly vectors in Abu Hamed focus was measured in this study. Entomological surveys for aquatic stages and adult *Simulium hamedense* were conducted before and after the inception of Merowe Dam in 2007/2008 and 2010/2011. There was no black fly breeding or adult activity in the previously known breeding sites upstream of the Merowe Dam with the western most breeding site found in Al Sarsaf village near the center of the focus. No adult or aquatic stages of black flies were found downstream of the Dam. The artificial lake of the Dam flooded all the breeding sites in the western region of the focus and no aquatic stages and/or adult black fly activity were established in the study area upstream of the Dam. The Dam seems to have positive impact on onchocerciasis and its black fly vectors in Abu Hamed focus. These outcomes of the Merowe Dam might have contributed to the recently declared interruption of onchocerciasis transmission in Abu Hamed focus. Continuous entomological surveys are needed to monitor presence of black fly vectors and its impact on the disease.

1707

THE CURRENT STATUS OF LYMPHATIC FILARIASIS IN COTE D'IVOIRE PRIOR TO IMPLEMENTATION OF A NATIONAL PROGRAM OF MASS DRUG ADMINISTRATION

Aboulaye Meite[1], Andrew C. Majewski[2], Gary J. Weil[2], Peter U. Fischer[2]

[1]Programme National de lutte contre la Schistosomiase, les Géohelminthiases et la Filariose Lymphatique, Ministry of Health, Abidjan, Côte D'Ivoire, [2]Washington University School of Medicine, St. Louis, MO, United States

Côte d'Ivoire is planning to implement a coordinated national program of mass drug administration (MDA) for elimination of onchocerciasis (Oncho) and lymphatic filariasis (LF) in the near future. The country has complex patterns of endemicity for these infections with extensive areas of coendemicity, areas that have received mass drug administration with ivermectin for variable periods, and extensive migration to and from neighboring countries (Liberia, Ghana, and Burkina Faso). For example, some areas in the northern part of the country have received many rounds of ivermectin for onchocerciasis control during OCP and APOC, and LF rates tend to be low in the North. In contrast, Oncho is uncommon in coastal areas (with some exceptions), and little ivermectin has been used in the South. Some 47 of the country's 82 health districts (mainly in Central and Southern districts) are considered to be co-endemic for LF and Oncho. LF mapping circa 2001 was based on antigen testing (Binax Now Filariasis, card test) of 50-100 people in two villages per district. The current study was performed to obtain more current information on the distribution of LF in the country and to identify sentinel sites for monitoring and evaluation of the impact MDA on LF. More than 3,900 people were tested for filarial antigenemia in 40 villages in 6 districts in the central and Southeastern part of the country. Antigen rates ranged from 4-22% in Lakota, 4-21% in Tiebissou, 15-41% in Akoupe, 21-25% in Agboville, 9-14% in Bettie, and 6-35% in Abengourou districts. Microfilaremia rates ranged from 1% in Lakota to 11% in Abengourou and Agboville. Prior ivermectin distribution in areas with coendemic onchocerciasis may partially explain the highly variable Mf rates in these areas. This study has helped to establish the current LF situation in Côte d'Ivoire, and this information will be used to plan and implement the national LF elimination program based on MDA.

1708

PROGRAMMATIC IMPLICATIONS OF EXTENSIVE VECTOR CONTROL ON THE ELIMINATION OF LYMPHATIC FILARIASIS IN ZAMBIA

Mutale Nsakashalo[1], Michelle Stanton[2], Enala Mwase[3], Elizabeth Chizema[1], Victor Mukonka[4], Peter Songolo[5], Brent Thomas[2], Maria Rebollo[2], Moses Bockarie[2], J. Russell Stothard[2], Louise Kelly-Hope[2]

[1]Ministry of Health, Lusaka, Zambia, [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [3]University of Zambia, Lusaka, Zambia, [4]Copperbelt University of Zambia, Ndola, Zambia, [5]World Health Organization Country Office, Lusaka, Zambia

Lymphatic filariasis (LF) is widely endemic in Zambia, and the National LF Programme is planning the first nationwide mass drug administration (MDA) to interrupt transmission in 2014. Overall the LF prevalence is low (<10%) in most regions of the country and it is possible that the well establish vector control programme for malaria has already impacted LF transmission over the past decade. To better understand the distribution of vector control that has occurred across the country and the potential implications for the LF Programme as it moves to scale up MDA, this study developed a spatially modelled vector control map and examined it in relation to the baseline LF prevalence data collected across 108 geo-referenced sites in 2003 and 2011, and the sentinel site data collected across 32 geo-referenced sites in 2014. Information on bed nets, including long-lasting/insecticide treated bed nets (LLIN/ITNs) and indoor residual spraying (IRS) distributions was obtained from the Ministry of Health, and public data sources such as the Demographic Health Survey (DHS) data, President's Malaria Initiative (PMI) reports, and combined in a weighted sum to form a multiple vector intervention score, which was then used to produce district-level maps of vector control intensity. Each district was classified according to LF prevalence and the vector intervention score which included the following combinations i) low LF /high vector control, ii) low LF / low vector control iii) high LF/ high vector control and iv) high LF/low vector control. These groups will help the LF Programme as it scales up MDA to determine if a district has potential for elimination, in need of very high MDA coverage and will require standard or enhanced surveillance.

1709

MODELING IMPACTS OF INTEGRATED VECTOR CONTROL ON LYMPHATIC FILARIASIS TRANSMISSION DYNAMICS AND ELIMINATION PROCESS

Brajendra K. Singh, Edwin Michael

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

Lymphatic filariasis (LF) is a target for global eradication by 2020. Launched in 2000, the Global Programme to Eliminate Lymphatic Filariasis relies mainly on large-scale preventative chemotherapy programs via mass drug administration (MDA) to eliminate this major vector-borne parasitic disease from all endemic settings. Modeling studies of LF transmission and control have been crucial for designing national control programs by establishing and quantifying the presence of infection and vector biting thresholds below which the transmission is interrupted. Renewed malaria control efforts have witnessed large-scale applications of vector control

(VC) through the use of long lasting insecticidal nets (LLIN) alone or in some combination with indoor residual spray (IRS) across the majority of LF endemic regions. LLIN/IRS affects the transmission of mosquito-borne infections either by directly killing, or by preventing mosquitoes from coming into contact with infected hosts through several mechanisms. Recent community trials have shown the substantial impact that VC may have in enhancing LF transmission interruption particularly when infection prevalence has been depressed to low levels using MDA. Despite these observations, theoretical and quantitative modeling of the impact of VC on LF transmission dynamics that takes explicit account of the various effects the different VC options may have on mosquito populations is scarce. Such analysis is vital when chemical insecticides require repeated applications in the affected communities due to their variable durations of effectiveness. This need for frequent insecticide applications introduces a number of factors such as the effects of adherence to the recommended timeframe for the replenishment of LLIN/IRS and the maintenance of the required community coverage, which may contribute to different outcomes from VC between communities. We aim to extend our present Bayesian Melding LF modeling framework by incorporating the specific effects that the application of LLIN and IRS, used separately or in combination, may have on the effective mosquito biting rate to quantify and gain better insights on the role of VC in the MDA-based LF control programs.

1710

AXENICALLY-DERIVED *CAENORHABDITIS ELEGANS* ANTIGEN FOR THE TREATMENT OF AUTOIMMUNITY

Belinda M. Jackson[1], Marina N. Torrero[2], Edward Mitre[1]

[1]Uniformed Services University, Bethesda, MD, United States, [2]National Institutes of Health, Bethesda, MD, United States

The potential use of helminth infections as a protective measure against inflammatory disease has been validated in both animal and clinical studies. However, several obstacles impede the use of parasitic helminths in clinical practice. These include difficulties in obtaining large quantities due to complex lifecycles, challenges in purification from lifecycle hosts, inter-batch variability in product, and the potential of live infections to cause clinical symptoms. To overcome these hurdles, we tested whether a homogenate of soluble antigens prepared from axenically grown *Caenorhabditis elegans* (aCeAg) would protect against autoimmunity. Using a TLR4 reporter cell line, we demonstrated that, in contrast to soluble antigen prepared from *C. elegans* grown on *Escherichia coli* lawns, aCeAg lacks LPS and does not activate TLR4. Twice weekly intraperitoneal injections of 100 mcg of aCeAg protected against the development of type 1 diabetes in non-obese Diabetic (NOD) mice (80% T1DM in PBS-injected controls, vs 10% in aCeAg group). Histological analysis demonstrated twice as many pancreatic islets in aCeAg-treated mice ($p<0.001$) as well as greater numbers of uninfiltrated islets. As observed in studies using antigens from parasitic helminths, aCeAg treatments increased the levels of basophils, eosinophils, and polyclonal and helminth-specific IgE immunoglobulins. Further, we observed increased production of the suppressive cytokine IL-10 ($p<0.05$), but not of the pro-inflammatory cytokine IFN-gamma, from splenocytes of aCeAg-treated animals. This study demonstrates proof-of-concept that antigens obtained from the non-parasitic nematode *C. elegans* can be used to obtain the same immune responses, and same immunoprotective effects, as parasitic helminths. Given that *C. elegans* can be grown axenically in controlled conditions without the need of any intermediate hosts, aCeAg may be able to overcome many of the current obstacles facing helmintic therapies for inflammatory diseases.

1711

DIFFERENCES IN OV-16 ELISA IMMUNE RESPONSES AMONG CHIMPANZEES INOCULATED WITH *ONCHOCERCA VOLVULUS*

Circe E. McDonald, Paul N. Cantey, Vitaliano Cama, Mark L. Eberhard

Centers for Disease Control and Prevention, Center for Global Health, Atlanta, GA, United States

Onchocerciasis, also known as river blindness, is a neglected tropical disease caused by the filarial parasite *Onchocerca volvulus*. Detection of immunoglobulin G4 (IgG4) antibodies to Ov16 recombinant antigen is the basis for serological tests for onchocerciasis. However, the dynamics of this immune response have yet to be thoroughly characterized. A non-human primate model was used to evaluate the temporal evolution of antibody responses under controlled infection conditions. Nine chimpanzees were inoculated with third-stage larvae of *O. volvulus*. Three chimpanzees were inoculated with approximately 100 stage three larva (L3) of Guatemalan origin, either one, three, or five times. Six chimpanzees each were inoculated once with 200, 300, or 400 L3 of Liberian origin. On a monthly basis, serum was collected and the presence of microfilariae (Mf) was determined via skin snip microscopy. Seven of nine chimpanzees developed patent infections, and six were used to evaluate the temporal responses over a median number of 1,660 days post-inoculation (PI). The seventh chimpanzee with patent infection was not evaluated due to health complications and was withdrawn at 535 days PI. Infections were categorized based on average microfiladermia of three consecutive dates as: weak (<10Mf/snip), mild (≥10 and <20) and strong (≥20 Mf/snip). One chimpanzee had a weak infection, two developed mild infections, and three had strong infections. No positive IgG4 responses to Ov16 were detected in the two inoculated but uninfected chimpanzees. The mean time to develop IgG4 responses and detection of Mf were 414 and 485 days PI. Four chimpanzees showed decreases in IgG4 values towards the end of the study. In three of these chimpanzees, decreased IgG4 responses were detected with decreasing microfiladermia. These findings indicate that positive serology to Ov16 occurs only among chimpanzees that developed patent infections, and suggest that anti-Ov16 antibody responses may decrease over time after reductions in detectable Mf loads in skin snips.

1712

CHARACTERIZING REACTIVITY TO *ONCHOCERCA VOLVULUS* ANTIGENS IN MULTIPLEX BEAD ASSAYS

Karla Feeser[1], Vitaliano Cama[1], Elizabeth A. Thiele[1], Thomson Lakwo[2], Sindew Mekasha[3], Jeffrey Priest[1], Paul T. Cantey[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Ministry of Health, Vector Control Division, Kampala, Uganda, [3]Ethiopian Public Health Institute, Addis Ababa, Ethiopia

Onchocerciasis is a neglected tropical disease targeted for elimination in Africa by 2025. Multiplex assays can provide a powerful platform for monitoring and evaluation, as well as integrated surveillance of onchocerciasis and other co-endemic diseases; however, the specificity and sensitivity of *O. volvulus* antigens have not been well-characterized within this context. A multiplex immunoassay was developed and used to evaluate three antigens (Ov16, Ov17 and Ov33) for onchocerciasis. The performance of each antigen was characterized using a panel of 499 specimens. One hundred ten samples were positive for onchocerciasis by skin snip microscopy and PCR, while the 389 controls were from people living in areas where onchocerciasis was not endemic and had infection with *Wuchereria bancrofti, Brugia malayi*, *Loa loa*, *Mansonella spp*, *Strongyloides stercoralis*, *Hymenolepis nana*, cysticercosis, schistosomiasis, or other human pathogenic parasites. All samples were analyzed in duplicate for IgG and IgG4 reactivity. Receiver Operator Characteristics (ROC) analyses were used to determine optimal cutoffs for all antigens. High sensitivity and specificity were detected for Ov16 and Ov33, while the Ov17 assays had specificities below 80%, identifying 75 false positives

among controls with lymphatic filariasis (LF). The Ov16 cutoff values for IgG or IgG4 were 379 and 32 fluorescent units (MFI), with sensitivities of 96.3 and 96.3% and specificities of 98.7 and 99.7%, respectively. For Ov33, a cutoff of 5,216 MFI in IgG reactivity resulted in 90.8% sensitivity and 97.2% specificity. The IgG4 cutoff was 67 MFI with a higher sensitivity of 96.3% and specificity of 98.5%.The IgG4 assay for both Ov16 and Ov33 detected few false positives, although the Ov33 assay detected 5 additional false positives among onchocerciasis-negative samples that were positive for either LF (3) or schistosomiasis (2). While no statistical difference was detected between the IgG and IgG4 assays for Ov16 and Ov33 (p>0.3), assays with the highest specificity and lowest cutoff values will help to ensure the ability of programs to monitor their work towards reaching desired elimination endpoints. Overall, Ov16 and Ov33 are highly sensitive and specific antigens in the multiplex platform. Further analysis of these antigens, either alone or in combination, may be useful for monitoring and evaluating progress towards the elimination of onchocerciasis.

1713

EVALUATION OF HLA IMMUNOINFORMATICS FOR THE IDENTIFICATION OF *BRUGIA MALAYI* PUTATIVE T CELL EPITOPES CONSERVED WITH *WUCHERERIA BANCROFTI* AND *LOA LOA*

Melissa Torres[1], Nils Pilotte[1], Tomabu Adjobimey[2], Sabine Specht[2], Frances Terry[3], Alexander Debrah[4], Linda Batsa[5], Alexander Kwarteng[2], Anne De Groot[3], Steven Williams[1], Achim Hoerauf[2]

[1]Smith College, Northampton, MA, United States, [2]Institute of Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Bonn, Germany, [3]EpiVax, Providence, RI, United States, [4]Faculty of Allied Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [5]Kumasi Centre for Collaborative Research in Tropical Medicine, Kumasi, Ghana

The availability of filarial genome sequences and improved immunoinformatics tools promises to accelerate the identification of highly conserved and immunogenic filarial vaccine components. The work done in this study has taken advantage of the functional similarities, rather than genetic diversity, of HLA binding residues in efforts to further identify putative T cell epitopes as potential vaccine antigens to combat lymphatic filariasis (LF). Predictions were previously made using the iVAX websuite containing both the EpiMatrix and ClustiMER immunoinformatics tools. 20-mer peptide sequences were selected for peptide synthesis from proteins within the *Brugia malayi* secretome. The 20 sequences were selected based on predictions to bind up to 8 of the most common HLA alleles represented within the software toolkit: DRB1*0101, DRB1*0301, DRB1*0401, DRB1*0701, DRB1*0801, DRB1*1101, and DRB1*1501. *In silico* filarial conservation analyses were done using basic local alignment tools within BROAD Institute's Filarial Worms Database. These analyses identified cross conservation for 6 out of the 20 sequences that shared sequence identity between *B. malayi* and *Wuchereria bancrofti* and/or *Loa loa.* Proof-of-principle assays for all 20 putative epitopes were performed using rHLA binding assays tested with 4 out of the 8 HLA alleles predicted by the software: DRB1*0101, DRB1*0401, DRB1*1101, and DRB1*1501. Results from these competitive binding assays demonstrated allele-specific binding biases. The 6 putative sequences sharing conservation with *W. bancrofti* and/or *L. loa* were tested on PBMCs from patients living in LF endemic areas that had been exposed to *W. bancrofti.* Upon peptide stimulation, subset CD4+ and CD8+ T cell populations from patients infected with *W. bancrofti* were selected for determination of cytokine-specific responses by ELISpot and flow cytometry. Results demonstrated the predicted peptides derived from the *B. malayi* secretome were capable of inducing T cell responses, which differed dependent on infection and disease status. These results suggested that the cross- conserved peptides were capable of binding to HLA from patients exposed to *W. bancrofti.*

1714

HOOKWORM INFECTION IN SCHOOL-AGED KENYAN CHILDREN IS ASSOCIATED WITH LOWER PHYSICAL FITNESS

Monica Nayakwadi Singer[1], Indu Malhotra[2], Francis Mutuku[3], Jackson Muinde[4], Peter Mungai[4], Dunstan Mukoko[4], Ginny Gildengorin[1], Charles King[2], A. Desiree LaBeaud[1]

[1]University of California San Francisco Benioff Children's Hospital Oakland, Oakland, CA, United States, [2]Case Western Reserve University, Cleveland, OH, United States, [3]Technical University of Mombasa, Mombasa, Kenya, [4]Division of Vector Borne and Neglected Tropical Diseases, Nairobi, Kenya

Children living in parasite-endemic resource-limited areas are heavily burdened by infection and the comorbidities associated with these diseases. Reduced physical fitness can result from anemia, undernutrition and chronic parasitic infections including soil transmitted helminths (STH), *Entamoeba histolytica*, *Giardia lamblia*, malaria and schistosomiasis. Our goal was to determine the prevalence of parasitic infections and their association with physical fitness as measured by the validated multi-stage 20 meter shuttle run (20mSRT) method. From January to March 2014, a cohort of 101children aged 4-7 years in coastal Kenya was evaluated. At the visit, blood, stool, and urine were collected and tested for presence of infection as follows: blood smear for malaria, Ritchie stool examination for STH, *E. histolytica*, and *G. lamblia*, and urine filtration for *S. haematobium*. 20mSRT were scored based on level achieved. Descriptive statistics were used to estimate infection rates. Wilcoxon scores determined the association between each type of infection and 20mSRT level achieved. The cohort included 101 children with a mean age of 5.8 years, 53% male. 43% reached 20mSRT level 1, 48% reached level 2, 5% reached level 3 and 4% reached level 4. Age, sex, and hemoglobin level (mean 10g/dL) were not significantly associated with the shuttle run level achieved. *Trichuris* was the most prevalent parasitic infection (13%), followed by hookworm (7%), *E. histolytica* (7%), giardia (5%), malaria (4%), *Ascaris* (1%), and schistosomiasis (1%). Hookworm infection was associated with a lower level achieved in the 20mSRT (p=0.002, 95% CI 0.014-0.113). Parasitic infections are common in school-aged children in coastal Kenya and may impair physical fitness. Hookworm infection, in particular, is associated with decreased physical fitness as measured by the 20mSRT.

1715

INFERENCE OF PARASITE BURDEN FROM INDIRECT INTENSITY DATA AND IMPLICATIONS FOR STUDY DESIGN

James E. Truscott, Arminder Deol, Roy Anderson

Imperial College London, London, United Kingdom

A substantial proportion of data collected in monitoring and evaluation (M&E) of macro-parasites records secondary indicators of the infection, such as egg counts or microfilaria, as a measure of parasite burden. Arguably, however, the most useful information for diagnosis, estimation of morbidity and for understanding the transmission dynamics of disease are quantities such as mean adult parasite burden and levels of aggregation of this burden within the population. Since adult worm counts are often very difficult to obtain, and we have only a few studies from which to estimate the relationship between adult worms and transmission stages, we need robust statistical descriptions of the distribution of measured quantities, such as egg counts, in terms of basic parameters of parasite biology. These will enable reliable inference of quantities such as mean worm burden, parasite aggregation and density-dependent processes from standard M&E data. We have developed a method for doing this calculation using extensive egg count and worm count data to identify and parameterize models of the distribution of Ascaris lumbricoides egg production as a function of worm burden. Results show that egg counts are best described by a negative binomial distribution with mean egg production from individual subject to an exponentially decreasing fecundity. Models of this kind will allow the optimal use of available data sources to extract reliable estimates of

basic biological parameters and their associated confidence intervals. This has strong implications for the tailoring of study design and choice of diagnostic techniques to optimize information gained against cost incurred. As an example, we discuss the reappraisal of M&E as elimination is approached and egg intensities fall.

1716

EFFECT OF A SINGLE DOSE OF 8 MG MOXIDECTIN OR 150 μG/KG IVERMECTIN ON INTESTINAL HELMINTHS IN PARTICIPANTS OF A CLINICAL TRIAL CONDUCTED IN NORTHEAST DRC, LIBERIA AND GHANA

Simon K. Attah[1], Kambale Kataliko[2], Maurice M. Nigo[3], Mawolo Kpawor[4], Nicholas O. Opoku[1], Eric Kanza[2], Didier Bakajika[3], Hayford Howard[5], Germain Mambandu[3], Kambale Kasonia[2], Michel Vaillant[6], Christine Halleux[7], Piero L. Olliaro[7], **Annette C. Kuesel**[7]

[1]Onchocerciasis Chemotherapy Research Center, Hohoe, Ghana, [2]Centre de Recherche Clinique de Butembo, Université Catholique du Graben, Butembo, Democratic Republic of the Congo, [3]Centre de Recherche en Maladies Tropicales de l'Ituri, C.R.M.T- ITURI, Hôpital Général de Référence de Rethy, Rethy, Province Orientale, Democratic Republic of the Congo, [4]The Liberian Institute for Biomedical Research, Clinical Research Center Bolahun, Monrovia, Liberia, [5]The Liberian Institute for Biomedical Research, Clinical Research Center Bolahun, Lofa County, Liberia, Monrovia, Liberia, [6]CCMS CRP-Santé, Luxembourg, Luxembourg, [7]World Health Organization, Geneva, Switzerland

In the Phase 3 study comparing the effects of a single dose of 8 mg moxidectin vs. ivermectin standard dose (150μg/kg) on *O. volvulus* skin microfilariae levels and participant well-being in 941 males and 531 females, including 79 children 12-17 years, , participants were randomized in a 2:1 ratio to moxidectin : ivermectin. 1465 participants underwent a single sample Kato-Katz test at screening. 876/1465 (60%) were infected with ≥1 type of intestinal helminth: *Ascaris lumbricoides* 44 (3%), *Trichuris trichuria* 22 (1.5%), hookworm 783 (53.4%), *Schistosoma mansoni* 233 (15.9%), *Strongyloides* 4 (0.3%). 835 with ≥1 type of intestinal helminth were treated with either moxidectin or ivermectin and tested again 1 month later with a single sample Kato-Katz test. Results obtained are expressed as cure rate (CR) and egg reduction rate (ERRam) using arithmetic mean egg counts post treatment relative to arithmetic mean egg counts pretreatment (EPG) for each species. Results for ivermectin treated subjects: *A. lumbricoides* n=10, EPG=408, CR=100%, ERRam=100%; *T. trichuria* n=6, EPG=2856, CR=83%, ERRam=76%; hookworm n=259, EPG=842, CR=29%, ERRam=52%; *S. mansoni* n=67, EPG=236, CR=54%, ERRam=73%. Results for moxidectin treated subjects: Ascaris lumbricoides n=34, EPG=386, CR=97%, ERRam=97%; *T. trichuria* n=11, EPG=1409, CR=91%, ERRam=99%; hookworm n=491, EPG=601, CR=48%, ERRam=82%; *S. mansoni* n=143, EPG=168, CR=64%, ERRam=66%. Co-administration of either ivermectin or moxidectin with drugs like benzimidazoles and/or praziquantel may help achieve high efficacy in preventive chemotherapy programmes for soil-transmitted nematodes and schistosomiasis.

1717

EPIDEMIOLOGY OF ANTHELMINTHIC TREATMENT FAILURE IN HOOKWORM (*NECATOR AMERICANUS*) INFECTIONS IN CHILDREN IN THE KINTAMPO NORTH MUNICIPALITY, GHANA. II. BASELINE PARASITOLOGY DATA

Michael D. Wilson[1], Josephine Quagraine[1], Gertrude Ecklu-Mensah[1], Joseph Otchere[1], Yvonne Ashong[1], Millicent Opoku[1], Lisa Harrison[2], Debbie Humphries[3], Kwadwo A. Koram[1], Michael Cappello[2]

[1]Noguchi Memorial Institute for Medical Research, Legon-Accra, Ghana, [2]Yale School of Medicine, New Haven, CT, United States, [3]Yale School of Public Health, New Haven, CT, United States

Previous studies in multiple communities in the Kintampo North Municipality (KNM), Ghana in 2007 revealed a high prevalence of hookworm infection (45%) and albendazole (single dose, 400mg) treatment failure (39%) school age children. Subsequent study in 2010 confirmed the high prevalence of hookworm (39%) and an even higher rate of albendazole treatment failure (56%) in this population. The overall goal of an NIH-funded longitudinal study is to fully characterize the epidemiology and molecular basis of benzimidazole treatment failure in KNM. Parasitological, anthropometric, socioeconomic, nutritional and immune status data were obtained from a cohort of consented 273 school-aged children (48.1% females and 51.9% males). Fecal samples, demographic data were also obtained from three consented household members of participants in two communities. We report here, the baseline parasitology results. Stool and blood samples were analyzed for intestinal helminths (Kato-Katz) and malaria (RDT and microscopy) infections, and PCR used to identify the hookworm species. All the participants gave fecal samples and 260 donated blood, and those found infected treated with albendazole. Fifty-eight (21.2%) were infected and 16 of 46 (34.7%) failed treatment and the overall cure rate was 61.7%. The geometric mean of intensity infection was 376.1epg (± 890.5) at pre-treatment and was 57epg (± 46.3) post treatment. The fecal egg count reduction rate was 81.2%. Sixty nine hookworm specimens were all identified as *Necator americanus*. 76.54% (199/260) and 67.7% (176/260) were positive by RDT and microscopy respectively. Geometric mean of *Plasmodium falciparum* intensity was 1377.3 (± 3623.9) parasites/ml of blood. 16.6% (45/173) participants were co-infected with both parasites. Hookworm and malaria co-infection rate was 17.3% (45/260). The cross sectional survey revealed hookworm prevalence of 33.3% (46/138) among household members, and 56.9% (29/51) of households, and the percent positive child with at least one household member positive was 73.3% (11/15).

1718

EPIDEMIOLOGY OF HOOKWORM (*NECATOR AMERICANUS*) INFECTIONS IN CHILDREN AND ANTHELMINTHIC TREATMENT FAILURE IN THE KINTAMPO NORTH MUNICIPALITY, GHANA. III. EVALUATION OF NUTRITIONAL RISK FACTORS AT BASELINE

Sena Apeanyo

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

An ongoing NIH-funded longitudinal study of school-age children aims to fully characterize the epidemiology of hookworm in the Kintampo North Municipality (KNM) where previous studies have found high albendazole treatment (400mg) failures in children. This arm of the study is to investigate the influence of modifiable host factors including overall nutritional status,food security and dietary diversity as risks factors of hookworm infestationat baseline. Structured questionnaires and food frequency questionnaires were used to collect data from 271 study participants. Haemoglobin concentration, weights and height z- scores were used to determine nutritional status of the study participants, WHO criteria was used to determine food security and food diversity. Fifty-eight participants (21.2%) and sixteen out of 46 studied participants (34.8%) were hookworm infected pre- and post-treatment respectively.

The overall mean Hb levels(anemia defined as <12.0g/dl) was 11.47g/dl (±1.28), 11.50g/dl (±1.25)and 11.35g/dl (±1.38) for negative and positive cases respectively, which were not significantly different between them. The mean weight z-scorefor the study participants was -0.02 (±0.97), 0.65 (±1.19) and -0.39 (±0.89) for negative and positive cases respectively, which were significantly between the two groups (P=0.02). The mean height z-score overall, was -0.01 (±1.0), -0.01 (±0.97)and -0.12 (±1.07) for negative and positive cases respectively, which were not significantly between the two groups (P=0.087). 74.2% of the study participants experienced food insecurity and so were 72.4% and 74.6% of the negatives and positives cases respectively. Above-average dietary diversity was observed in 46.5% of the study participants, and was 48.8% and 37.9% for negative and positive cases respectively. In conclusion, most of the study participants were anemic, underweight, stunted and consumed less diverse food. Moreover, infected children were significantly underweight than their non-infected counterparts.

1719

COMPARING METHODS TO ASSESS *SCHISTOSOMA* RESPONSE TO PRAZIQUANTEL TREATMENT

Piero L. Olliaro[1], Michel Vaillant[2], Aïssatou Diawara[3], Jean T. Coulibaly[4], Amadou Garba[5], Jennifer Keiser[5], Charles H. King[6], Stefanie Knopp[5], Aly Landouré[7], Eliézer K. N'Goran[4], Giovanna Raso[5], Alexandra U. Scherrer[8], José Carlos Sousa-Figueiredo[9], Katarina Stete[5], Xiao-Nong Zhou[10], Jürg Utzinger[5]

[1]World Health Organization, Geneva, Switzerland, [2]Methodology and Statistical Unit, Centre for Health Studies, CRP-Santé, Luxembourg, Luxembourg, [3]New York University Abu Dhabi, Abu Dhabi, United Arab Emirates, [4]Unité de Formation et de Recherche Biosciences, Université Félix Houphouët-Boigny, Abidjan, Côte D'Ivoire, [5]Department of Epidemiology and Public Health, Swiss Tropical and Public Health Institute, Basel, Switzerland, [6]Center for Global Health and Diseases, Case Western Reserve University, Cleveland, OH, United States, [7]Institut National de Recherche en Santé Publique, Bamako, Mali, [8]Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zürich, University of Zürich, Zürich, Switzerland, [9]Department of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, United Kingdom, [10]National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, Shanghai, China

To diagnose *Schistosoma* infection, stool or urine samples are examined for worm eggs, but there is no definitive agreement as to how best express treatment efficacy. We analyzed data from 24 trials conducted in Africa, Asia and Latin America enrolling overall 4740 individuals infected with *S. mansoni* (Sm, n=1804; 38.1%), *S. haematobium* (Sh, n=2633; 55.5%), or *S. japonicum* (Sj, n=303; 55.5%), and treated with praziquantel at doses of 40 (n=3713), 60 (n=690) and 80 (n=337) mg/kg. Efficacy was measured using cure rate (CR) and egg reduction rate (ERR) calculated using either geometric or arithmetic means (ERRgm, ERRam). We compared efficacy outcome measures: 1) ERRam vs. ERRgm, 2) ERR vs CR, and 3) ERR and CR based on quadruplicate vs single Kato-Katz thick smear examination for Sm. We found that: 1) ERRam and ERRgm can be used interchangeably only if treatment efficacy is very high (>95%); as efficacy falls, estimates are higher with ERRgm than ERRam. Modeling data shows that consistency between means is better for Sh and Sj than for Sm; 2) poor correlation between ERRgm/am and CR except when ERRs are very high (>97%). 3) using a single rather than quadruplicate Kato-Katz thick smear excluded 19% of Sm-infected individuals; the effect on estimating ERR was negligible by individual studies; however, on aggregate ERRam and CR were 8-9% higher (no effect on ERRgm.) A valid complement for drug efficacy monitoring is to study the distribution of individual responses to identify suboptimal responders. Of the 2358 Sh-infected individuals with complete data records 61.3% were negative post-treatment (cure rate, CR), 32.4% had reduced egg counts (rEC), 6.3% had no change/increased egg counts (nEC). For Sm (n=1699) individuals CR was 75.4%, rEC 20.5%, nEC 4.1%. For Sj (n=300) CR was 90%, eEC 8.3%, nEC 1.7%. The response achieved by the 5th centile (the 5% worse responders) was 79.1%, 77.9%, and 23.6% for Sj, Sh and Sm; for the 10th centile it was 100%, 88.2%, and 70.3%, and for the 25th centile 100%, 97.8%, and 100%, respectively.

1720

EPIDEMIOLOGY OF HOOKWORM (*NECATOR AMERICANUS*) INFECTIONS IN CHILDREN AND ANTHELMINTHIC TREATMENT FAILURE IN THE KINTAMPO NORTH MUNICIPALITY, GHANA. I. BASELINE INDICATORS OF EPIDEMIOLOGY AND SOCIOECONOMIC RISK FACTORS

Hector L. Samani[1], Sena Apeanyo[1], Karen Zhang[2], Debbie Humphries[2], Kwadwo A. Koram[1], Michael Cappello[3], Michael D. Wilson[1]

[1]Noguchi Memorial Institute for Medical Research, Legon-Accra, Ghana, [2]Yale School of Public Health, New Haven, CT, United States, [3]Yale School of Medicine, New Haven, CT, United States

In 2007 and 2010, high prevalence rates of hookworm infections (45%) and (39%) respectively in school age children was observed in the Kintampo North Municipality (KNM), Ghana. Also observed were high rates of albendazole (single dose 400mg) treatment failure; (39%) and (54%) respectively. As part of an NIH-funded study which aims to fully characterize the epidemiology and molecular basis of benzimidazole treatment failure in KNM, a census/enumeration survey was conducted in eight communities in KNM, then 271 children aged between 8 and 12 years were randomly selected for a longitudinal study. To define the epidemiology of hookworm infection at baseline and the specific host factors associated with albendazole treatment failure, demographic, socioeconomic and environmental information was collected using structured questionnaires. The cohort comprised of 138 males and 133 females. The cohort's mean age was 9.49 (± 1.69). The overall hookworm prevalence was 21.4% (58/271), of which 67.2% of infected children were the group 10 years and above. Significant associations were found between hookworm infections and possession of cattle and dogs (P=0.049 and P=0.029), ownership of shoes (P=0.015) and wearing shoes daily (P=0.020). No significant associations were found with gender, age, access to agricultural land, and types of water sources and toilet facilities (P>0.05). No associations were also found between treatment response and any of the socio-economic parameters (P>0.05).

1721

RICE FORTIFIED WITH IRON AND OTHER MICRONUTRIENTS IMPACTS HOOKWORM INFECTION RISK IN SCHOOLCHILDREN

Brechje de Gier[1], Maiza Campos Ponce[1], Marlene Perignon[2], Marion Fiorentino[2], Khov Kuong[3], Chhoun Chamnan[3], Megan Parker[4], Kurt Burja[5], Jacques Berger[2], Katja Polman[6], Frank Wieringa[2]

[1]VU University Amsterdam, Amsterdam, Netherlands, [2]IRD, Montpellier, France, [3]DFPTQ, Fisheries Administration, Phnom Penh, Cambodia, [4]PATH, Seattle, WA, United States, [5]WFP, Phnom Penh, Cambodia, [6]ITG, Antwerp, Belgium

Soil-transmitted helminth (STH) infections often co-exist with micronutrient deficiencies in the developing world. There is evidence that these major health issues can influence and exacerbate each other. Fortification of staple foods such as rice can be an effective tool to improve micronutrient status of vulnerable groups, but its impact on STH infection is currently unclear.In a cluster randomized, placebo-controlled, double-blinded trial, 3 different types of fortified rice were introduced through the World Food Program (WFP) School Meal program in Cambodia. The 3 types of fortified rice differed in micronutrient compositions and production method. Children (6-15 y) received 1 type of rice, unfortified rice (placebo), or no school meal (control) for 7 months. Stool samples were collected and analyzed by Kato Katz method at baseline, 3 months and 7 months. After baseline, all children received a single dose of 400mg albendazole.

The effects of consumption of fortified rice on hookworm infection were analyzed by multiple logistic regression. Baseline prevalence of STH was 17.0%, which were mainly hookworm infections (16.6%) of light intensity. A risk factor for hookworm infection was being a boy (P=0.011). After 7 months (n= 1236 children), hookworm infection prevalence was between 17-24% in control, placebo and in the NutriRice fortified rice groups. In the children receiving 2 types of UltraRice fortified rice, hookworm prevalence was 33-34% (P<0.001). The new infection rate was highest in the UltraRice group with the highest iron content (24.6%), intermediate in the UltraRice group with less iron (21.8%), and lowest in the control and placebo and groups (12.5% and 11.9%, P=0.001 for difference among groups). Fortifying rice with micronutrients, especially iron, can increase risk of hookworm infection. Type of fortificant appears to be a major effect modifier. These findings have big implications for policies aiming to improve child health and nutritional status in tropical regions.

1722

HOW DOES THE SCALE OF DEWORMING PROGRAMS AFFECT THEIR COST-EFFECTIVENESS?

Hugo C. Turner, James M. Truscott, Roy M. Anderson

Imperial College London, London, United Kingdom

The predominant control strategy for the soil-transmitted helminths (STHs) is regular periodic mass drug administration (MDA), targeting school-aged children. The World Health Organization (WHO) and London declaration on Neglected Tropical Diseases (NTDs) has set goals to scale up MDA, so that by 2020, 75% of the pre-school and school-aged children in need, will be treated regularly. It has been observed that increasing the number treated can reduce the per capita costs of MDA programmes (economies of scale). This is because a number of the costs associated with MDA delivery are fixed (i.e. do not depend on the number treated), and therefore increasing the number treated reduces the average fixed cost per treatment. However, the implications this has on the cost-effectiveness of scaling up control for STH infections, and the optimum treatment strategy have not been explored. We developed costing functions which account for the changes in the per capita costs of treatment with scale, and incorporated them into STH dynamic transmission models. We found that, due to these economies of scale, the cost effectiveness of STH control programmes markedly increased with the number treated. This has notable implications for programmes considering scaling up MDA, in line with the current goals set by the London declaration.

1723

THE EFFECT OF SEASONALITY ON THE PREVALENCE OF *ASCARIS LUMBRICOIDES* AND IMPLICATIONS FOR THE OPTIMAL TIMING OF MASS TREATMENT PROGRAMS

Emma L. Davis[1], Rachel Pullan[2], Leon Danon[3], Deirdre Hollingsworth[1]

[1]University of Warwick, Coventry, United Kingdom, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]Queen Mary University of London, London, United Kingdom

Previous studies have shown that changes in temperature affect maturation times, development proportions, and mortality rates of *Ascaris lumbricoides* and *suum* eggs, which suggests that climate could impact both overall prevalence and reinfection rates after treatment. Depending on the size of this effect, this could have implications for the optimal time of year for mass treatment in order to get the maximum impact out of the large number of donated drugs. However, there has been little quantitative investigation of the influence of seasonal timing of mass chemotherapy on treatment programmes. Using historical data from experimental studies on *A. suum* eggs – which have been shown to display equivalent behaviour to *A. lumbricoides* eggs – to characterise relationships between temperature and egg maturation and survival, models were developed which investigated the effect of temperature dependent development of eggs on mean worm burden in the human population. These reveal fast maturation and low egg mortality at high temperatures, but also a drop in proportion of eggs reaching maturity above 30°C. To demonstrate implications for the optimal timing of mass treatment campaigns, models were applied to districts with differing levels of *A. lumbricoides* transmission across Kenya. District-level prevalence estimates were generated using predictive risk maps developed by the Global Atlas of Helminth Infection and monthly temperature patterns were derived from MODIS. Results suggest timing of treatment could have important consequences for programme impact. Depending on region, changing the treatment date resulted in an estimated 18-55% comparative decrease in prevalence after four yearly treatment rounds. This highlights the potential importance of appropriate timing of the established Kenya National Deworming Programme. More generally, this approach provides insight into the epidemiology of *A. lumbricoides* infection, methods for testing and validating these predictions, and can help guide optimal long-term helminth control strategies in diverse settings.

1724

KEY RESIDUES OF CRY5B STRUCTURE AND FUNCTION: MUTAGENESIS BY ALANINE SCANNING

Jillian Sesar, Yan Hu, Hui Fan, Partho Ghosh, Raffi Aroian

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

Soil-transmitted helminthes infect more than 2 billion people worldwide and only one drug (albendazole) is able to show a high enough efficacy against parasite worms under conditions for mass drug administration. However, recent studies have shown an increase in resistance to this drug, stressing the importance of finding a new treatment option. Crystal (Cry) proteins produced from the soil bacterium Bacillus thuringiensis have been used for decades as a means to control insects that destroy crops and transmit human diseases, and studies have shown these proteins to be safe to humans. Our lab has shown that crystal proteins, specifically Cry5B, are able to kill both the free-living nematode Caenorhabditis elegans, as well as parasitic roundworms (eg. *Ancylostoma ceylanicum*, hookworm). Cry proteins intoxicate invertebrates by acting as pore-forming toxins. Several defined steps in their mechanism of action have been suggested from insect studies, but there is still great uncertainty as to the importance of these various steps. We believe that the nematode - Cry5B system has great potential to unlock mysteries surrounding Cry proteins and to be a potential therapeutic agent. Here, I have mutated all of the 698 amino acids in the toxin domain of Cry5B, and subsequently tested these mutants on C. elegans to assess for changes in toxicity levels, screening for variants with an increase in activity as compared to the wild type. From this screen and subsequent quantitative LC_{50} killing assays to confirm the screen results, I have identified several key variants of interest that are additionally more active against *A. ceylanicum* both *in vitro* and *in vivo*. Additionally, these residues most likely play a key role in Cry5B protein function, with the eventual goal being to correlate these changes in activity with specific changes in protein functionality. These improved Cry protein variant candidates have the potential to be used in therapeutics for treating one of the most neglected diseases of our time, parasitic worms.

1725

A NOVEL NEXT-GENERATION SEQUENCING APPROACH TO DEVELOP IMPROVED MOLECULAR DIAGNOSTICS FOR THE DETECTION OF SOIL TRANSMITTED HELMINTH INFECTIONS

Nils Pilotte, Marina Papaiakovou, Lou Ann Bierwert, Steven A. Williams

Smith College, Northampton, MA, United States

The soil transmitted helminths (STHs) are a group of parasitic worms responsible for causing extensive morbidity in many of the world's most economically depressed locations. With an estimated 880 million children infected with one or more species of STH parasite, accurate and cost-effective diagnostic measures are of premier importance to global control and elimination efforts. Accordingly, the use of molecular

diagnostic measures, such as real-time PCR, has shown great promise for the improved detection of STH infections. To date, such molecular assays have utilized ribosomal and mitochondrial target sequences, as previously reported. While effective, such sequences are frequently not the highest copy number target and will not yield the most sensitive assay possible. The most sensitive assay will utilize the most highly repetitive, unique, non-coding DNA sequences found within the genome of each species. Consequently, we have coupled next-generation sequencing technology with the Galaxy-based software *RepeatExplorer*, to identify the most numerous, non-coding DNA sequences within multiple species of STH parasites including *Trichuris trichiura*, *Necator americanus*, *Ancylostoma duodenale*, and *Ascaris lumbricoides*. Following the application of this approach to each STH species, we designed TaqMan-based primer-probe combinations for each candidate sequence. Species-specificity was verified for each assay and repeatable detection of genomic DNA isolated from each parasite was demonstrated at concentrations ranging from 1.0ng to 1.0fg. Through this novel approach to the identification of species-specific, high copy-number target sequences, we have developed a new strategy for the design of a PCR-based diagnostic assay with improved sensitivity.

1726

THE ROAD TOWARDS EFFICIENT CONTROL OF SCHISTOSOMIASIS IN THE DEMOCRATIC REPUBLIC OF CONGO: PRE-ASSESSMENT OF STAFF PERFORMANCE AND MATERIAL RESOURCES IN ENDEMIC REGIONS

Sylvie Linsuke[1], Liliane Mpabanzi[2], Joules Madinga[3], Sabin Nundu[1], Faustin Mukunda[4], Pascal Lutumba[1], Katja Polman[2]

[1]Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of the Congo, [2]Institute of Tropical Medicine, Antwerp, Belgium, [3]University of Kinshasa, Kinshasa, Democratic Republic of the Congo, [4]Programme National de Lutte contre la Bilharziose et Parasitoses Intestinales, Kinshasa, Democratic Republic of the Congo

Schistosomiasis is a disease affecting approximately 200 million people worldwide. Since a long time, schistosomiasis has been known to be endemic in certain provinces of the Democratic Republic of Congo. However, the most recent national data available on schistosomiasis prevalence and distribution were published in the early sixties. . Recently, the Ministry of Health adopted a national plan against schistosomiasis aiming at to distribute Praziquantel (PZQ), the treatment of choice for schistosomiasis, to all individuals infected. For effective introduction of control strategies, data on national prevalence and distribution of schistosomiasis in the DRC urgently need to be updated. The present study assessed the knowledge of health workers on schistosomiasis as well as the availability of the facilities needed for adequate diagnosis and management of the disease in the endemic provinces of Kinshasa and Bas-Congo in the DRC. This study was conducted in 9 health zones (HZ) of Kinshasa and 2 HZ in Bas-Congo. Health workers could name all symptoms of schistosomiasis. Kato-Katz, urine filtration or sedimentation were not available as diagnostic methods in any health facilities. Diagnosis therefore almost solely relied anamnesis. The knowledge on schistosomiasis did not differ between the rural Bas-Congo and urban Kinshasa. The fees for consultation, diagnostics and treatment were three times higher in Kinshasa than Bas-Congo. Health workers in Kinshasa and Bas-Congo are able to name the symptoms related to schistosomiasis. However there is a lack of availability of adequate diagnostic tools and treatment. The fees of diagnostics and treatment are high for a population often living in extreme poverty.

1727

HELMINTH INFECTIONS DURING PREGNANCY MAY DECREASE NUTRITIONAL FITNESS OF THE OFFSPRING

Emily A. McDonald[1], Sunthorn Pond-Tor[1], Blanca Jarilla[2], Marianne J. Sagliba[2], Analisa Gonzal[2], Remigio Olveda[2], Luz Acosta[2], Jonathan D. Kurtis[1], Jennifer F. Friedman[1]

[1]Rhode Island Hospital, Providence, RI, United States, [2]Research Institute of Tropical Medicine, Manila, Philippines

Helminth infections represent a significant disease burden in endemic regions of the world, and polyparasitism may have a bigger impact on overall health than any individual infection. We have previously shown that *Schistosoma japonicum* results in a profound pro-inflammatory response at the maternal-fetal interface during pregnancy and decreased invasion characteristics of placental trophoblast cells *in vitro*. Another critical function of the placenta is to regulate nutrient exchange between mother and fetus. Herein, we have shown that treatment *in vitro* of primary trophoblasts with schistosome soluble egg antigens (SEA), resulted in a significant drop in gene expression of specific amino acid transporters. These include the sodium-coupled neutral amino acid transporter 1 (SNAT1; 80% reduction) and large neutral acid transporter (LAT1; 70% reduction). To investigate the metabolic impact of helminth infections during pregnancy, we utilized samples from a cohort of pregnant women from Leyte, the Philippines. Most subjects had polyparasitic infections, including schistosomiasis and geohelminth infections, with prevalence rates of 70%, 79%, and 40% for *Ascaris lumbricoides, Trichuris trichiura,* and hookworm, respectively. Given the relatively low intensity of schistosome infection and the high prevalence rates of geohelminths, we assessed the relationship between the number of helminth infections and metabolic parameters *in utero*. After controlling for SES and gestational age, leptin levels were found to be lower in the cord blood of infants born to mothers with one or more helminth infections. In addition, cord blood leptin levels were positively associated with birth weight (107g heavier on average in those infants in the highest tertile of leptin levels), and increased leptin levels were associated with a reduced risk of fetal growth restriction. These data suggest that helminth infections can impact the transport of nutrients across the maternal-fetal interface, providing a possible link between fetal metabolic hormones and growth *in utero*.

1728

GUT INSTINCTS: EVALUATING PARENTAL ATTITUDES TOWARD INTESTINAL WORM TREATMENT IN RURAL CHINA

Louise Lu[1], Chengfang Liu[2], Alexis Medina[1], Darvin Scott Smith[1], Scott Rozelle[1]

[1]Stanford University, Stanford, CA, United States, [2]Center for Chinese Agricultural Policy, Beijing, China

Nearly forty percent of elementary schoolchildren in rural provinces of Southwest China are infected with soil-transmitted helminths. However, parasitic worm infection is neglected as a major public health problem in these villages and deworming treatment is rarely sought, despite its efficacy and low cost (one treatment dosage costs less than 3 cents USD). Surveys and interviews were conducted in six rural villages in Guizhou, China to evaluate what factors influence parental decisions to seek or not seek deworming among rural Chinese schoolchildren. It was found that knowledge about helminth infection and prevention was severely lacking and often influenced by deep-rooted myths, such as the local belief that deworming medicine can harm a child's future fertility. The majority of household interviewees were highly skeptical of high worm prevalence in their children, despite the nearly universal practice of regularly deworming their pigs. A comprehensive deworming program involving biannual administration of deworming treatment, household health education, and village health system strengthening is necessary to effectively mitigate the disease burden of helminth infection in rural China.

1729

RANDOMIZED CONTROLLED TRIAL OF TWO IVERMECTIN REGIMENS FOR *STRONGYLOIDES STERCORALIS:* EARLY FINDINGS

Guadalupe Jimenez[1], David Goodman-Meza[1], Herbert B. Tanowitz[2], Christina M. Coyle[2]

[1]Jacobi Medical Center, Bronx, NY, United States, [2]Albert Einstein College of Medicine, Bronx, NY, United States

Humans can be chronically infected with *Strongyloides stercoralis* for decades because of the mechanism of autoinfection. Although most infected individuals are asymptomatic, *S. stercoralis* is capable of transforming into a fatal illness in setting of HTLV-1 and steroids. Chronic infection with *S. stercoralis* presents both diagnostic and therapeutic challenges. Parasitologic diagnosis of chronic infection is difficult, because the larval output can be low and irregular. The diagnostic accuracy of serologic testing has a sensitivity and specificity of 93% and 95%, respectively. Ivermectin is the treatment of choice for this parasitic infection, although optimal dosing is yet to be determined. This study evaluated the serologic response to *S. stercoralis* infection after treatment with ivermectin 200 mcg/kg given 2 weeks apart based on the auto-infective cycle (Group A) vs. given on two consecutive days (Group B). Patients were referred from outpatient clinics or identified on the in-patient services and invited to participate in the study. Participants were randomized to either treatment arm and repeat serologies were performed at 3 months intervals for 9 months after treatment. Forty-seven cases were enrolled, mean age was 54.1 (SD 15.2), 61.7% male, 55.3% Hispanic, mean eosinophil count 0.55/nl (SD 0.53), IgE 608.0 mg/dl (SD 680.4) and HTLV-1 was negative in all cases. There were no significant differences in baseline demographic or clinical variables between the two groups. Of the 47, 51.0% had completed the 9 months follow-up. Mean eosinophil count (p=0.002) and IgE value (p=0.045) both decreased after treatment of cases in both treatment groups. Of the 47 patients, 9 cases remained sero-positive on follow-up; five (22.7%) in Group A and four (16.0%) in Group B (p=0.751). In this randomized controlled trial, there was no difference in serologic outcome in the two treatment arms, but treatment resulted in decreased eosinophil and IgE values.

1730

TRYPANOSOMA CRUZI INFECTION PREVALENCE AND BLOOD MEAL ANALYSIS IN VECTORS OF CHAGAS DISEASE IN SOUTHWEST TEXAS, 2013-2014

Rodion Gorchakov[1], Edward J. Wozniak[2], Teresa P. Feria[3], Melissa N. Garcia[1], Peter J. Hotez[1], Kristy O. Murray[1]

[1]Baylor College of Medicine, Houston, TX, United States, [2]Texas Department of State Health Services, Uvalde, TX, United States, [3]University of Texas Pan American, Edinburg, TX, United States

Protozoan pathogen *Trypanosoma cruzi* is an etiologic agent of Chagas disease, which affects millions of people in Latin America and is an emerging public health threat in the United States. Transmission cycle of the parasite involves alternating infection of its vertebrate hosts and insect vectors. We identified the current vector infection burden and potential range of natural reservoirs of *T. cruzi* in 11 southwestern counties of Texas by analysis of insects of genus *Triatoma* collected during the period from June 2013 to January 2014. Out of 40 submitted specimens, the vast majority of the insects were *T. gerstaeckeri*, with only four samples of *T. sanguisuga*, two samples of *T. lecticularia*, and one sample of *T. rubida*. We found 73% of the insects positive for *T. cruzi*. Blood meal analysis was performed on the infected triatomines. Blood sources were determined for all but one of the insects, and included 13 different species of mammals (mouse, woodrat, squirrel, porcupine, armadillo, cottontail, raccoon, fox, coyote, dog, pig, cow, human). Interestingly, 36% of the bugs were identified as having multiple blood sources. Since most of the insects were collected in or around residential houses, the most prevalent type of blood meal was human (50% of the insects). High infection rate of the triatomine vectors combined with high incidence of feeding on humans underscore the importance of Chagas disease surveillance in Texas and prompt for urgent measures for vaccine development, vector control, and increasing public awareness.

1731

POPULATION GENETIC STRUCTURE OF THE TSETSE FLY: TARGETING REPRODUCTIVE REFRACTORY INTERVENTIONS FOR WILDLIFE AND LIVESTOCK TRYPANOSOMIASIS IN KENYA

Benard Kulohoma

International Centre for Insect Physiology and Ecology, Nairobi, Kenya

Tsetse flies (*Glossina* species) are major vectors of both human and livestock trypanosomiasis, and efficiently transmit the causative parasites trypanosomes. Tsetse flies infest up to 10 million km^2 of land stretching across 40 countries in sub-Saharan Africa. Multiple drugs that treat African animal trypanosomiasis exist, and have substantially improved veterinary management of livestock with both susceptible and resistant trypanosome strains. Although, multi-drug resistant trypanosome strains have lower fitness and are therefore thought to be less persistent, the effect of increased communal use of multiple antibiotics on transmission rates of these pathogenic species is still not fully clear. Moreover, widespread multi-drug resistance due to prolonged usage or under-dosing could also have adverse repercussions on public health, further complicating management disease management. Our study, will exploit genomic approaches to understand the population structure of tsetse flies in circulation in disease endemic regions of Kenya so as to determine the impact of drug use on the prevalence of multi-drug resistance. It's effect on tsetse infectivity and transmissibility of multi-drug resistant trypanosome strains. This study also aims to identify genes essential for successful reproduction as potential long-term vector control targets. This study holds the promise of identifying socio-demographic independent vector control strategies, and will enable the judicious use of appropriate drugs to which trypanosome strains are not resistant. Thereby enabling prolonged trypanosome and tsetse control while avoiding widespread multiple-drug resistance.

1732

EMERGENCE OF A TROPICAL DISEASE IN U.S. DOGS: A PROSPECTIVE STUDY OF *LEISHMANIA* IN U.S. FOXHOUNDS

Angela J. Schneider, Robert Schaut, Christine Petersen

University of Iowa, Iowa City, IA, United States

Leishmania is the causative agent of leishmaniasis, a deadly protozoan disease which affects approximately 12 million people worldwide. Generally associated with tropical and subtropical regions, *Leishmania* can be found as far north as the United States. Similarly, the vector for *Leishmania* transmission, the sand-fly, has been found within areas as far north as Missouri and Ohio. *Leishmania infantum,* infects canines as well as humans. In endemic areas dogs serve as the domestic reservoir. Within the United States, foxhound hunt populations have developed endemic disease. The first documented foxhound case of *Leishmania* in the US was reported in 1980. *Leishmaniasis* is a chronic disease with long latency, diagnosis can be difficult with gold standards limited to invasive techniques including bone marrow aspirate with culture. Serological testing has been used as a diagnostic technique but has cross reactivity with *Trypanosoma cruzi* also found in the southern U.S. To help with diagnosis, a highly sensitive and specific real time quantitative polymerase chain reaction (RT-qPCR) assay was developed. While this assay is capable of identifying parasite DNA within the peripheral blood it lacks the ability to determine whether the *Leishmania* is actively proliferating. Over the course of the last 8 years, diagnostic testing using PCR increased. Cases of *Leishmaniasis* can be tracked throughout regions of the US within the foxhound hunt populations. This study is the first to report changes in *Leishmania* prevalence and incidence over a six-year span (2007-2012) within US foxhound hunts. Trends in infection over time and across regions

were examined. *Leishmania* infection has stayed consistent over time with a point prevalence in 2007 of 3.54 per 1000 foxhounds and 3.23 per 1000 foxhounds in 2012. Incidence rates over the 5-year period began at 4.77 per 1000 foxhounds in 2007 and ended at 3.08 per 1000 foxhounds in 2012. The consistent prevalence and incidence of this infection stresses the need for appropriate risk management and disease prevention techniques in this community.

1733

MATERNAL *TRYPANOSOMA CRUZI* INFECTION AND INFANT GROWTH

Elizabeth J. Howard[1], Xu Xiong[1], Maria Luisa Cafferata[2], Gabriela Cormick[3], Alvaro Ciganda[2], Pierre Buekens[1]

[1]Tulane University School of Public Health, New Orleans, LA, United States, [2]La Unidad de Investigación Clínica y Epidemiológica Montevideo, Montevideo, Uruguay, [3]Institute for Clinical Effectiveness and Health Policy, Buenos Aires, Argentina

Maternal *Trypanosoma cruzi* infection and subsequent congenital transmission is a serious, yet neglected, global health issue. Infant growth assessment can provide an understanding of the health of children and an indirect evaluation of the quality of life for an entire population afflicted with Chagas disease. The objective of this study was to determine if maternal *T. cruzi* infection indirectly affects a newborn's physical development. Infected mothers and their infants (n=153) were followed at birth, 4-8 weeks and 10 months post-partum in Tucuman, Argentina from April 2011 until April 2013. Age- and sex-specific estimates of infant weight, length, weight-for-length, and head circumference were compared to an international child growth standard. All mean z-scores were between ±1 standard deviations (SD) of the standard. However, the prevalence of infants falling below -2 SD of the WHO standard peaked at 16% for weight-for-age for females, 10.9% for length-for-age in males and 10.8% for weight-for-length in females, all at visit 1. Infants who experienced growth faltering were more likely to be female and weighed 0.8 kg less at birth, 0.9 kg less at 4-8 weeks and 0.5 kg less at 10 months of age. They also were 3 cm shorter and had a 2 cm reduction in head circumference. This analysis provides evidence of progressive stunting over the 10 month period and early failure to thrive with improvement by 10 months. Nutrition and health interventions, as well as socioeconomic changes, may be helpful in improving the growth and development of infants from Chagas affected populations.

1734

COMPARISON BETWEEN PATIENTS WITH CLASSICAL AND ATYPICAL PRESENTATIONS OF CUTANEOUS LEISHMANIASIS, FROM AN AREA OF *LEISHMANIA (VIANIA) BRAZILIENSIS* TRANSMISSION IN NORTHEAST BRAZIL

Luiz Henrique Guimarães, Adriano Queiroz, Juliana A. Silva, Silvana C. da Silva, Ednaldo L. Lago, Paulo Roberto L. Machado, Edgar M. Carvalho, **Albert Schriefer**

Hospital Universitário Prof. Edgard Santos, Salvador, Brazil

The purpose of this study was to compare clinical, immunological and parasitological aspects between patients with atypical cutaneous leishmaniasis (ACL) and classical cutaneous leishmaniasis (CL) of a region with high endemicity for American tegumentary leishmaniasis (ATL) in northeast Brazil. Fifty one ACL and an equal number of CL patients were enrolled. ATL was confirmed and parasite species was determined by PCR of lesion biopsies. For each patient clinical data was annotated, peripheral blood was collected, and parasite isolation was attempted. Cultured parasites were genotyped according to the sequence of a locus in chromosome 28, previously shown to be polymorphic in this population of *L. (V.) braziliensis*. All cases had their living sites geographic coordinates acquired by GPS then distributions of ACL and CL cases were compared by the Cusick and Edward's test (CE). Among ACL included there was no pregnant women or HIV positive subjects. ACL presented the same distribution as CL patients in the affected region (CE p = 0.26), but had a greater proportion of lesions above the waist line (94% in ACL x 33% in CL, p = 0.0001) and of failure to antimony treatment (41% in ACL x 0% in CL, p = 0.0006) than CL individuals. Immunologically, ACL showed lower production of TNFα (average 316.5 pg/ml in ACL x 1906.1 pg/ml in CL, p=0.0001) and IFNγ (average 747.1 pg/ml in ACL x 4445.9 pg/ml in CL, p=0.0002), but higher IL-10 (average 392.8 pg/ml in ACL x 171.9 pg/ml in CL, p=0.0006) and IL-17 (average 218.4 pg/ml in ACL x 69.4 pg/ml in CL, p=0.0008) after *in vitro* stimulation of peripheral blood mononuclear cells with leishmania antigen than CL patients. All subjects were infected with *L. (V.) braziliensis*, but parasites from ACL presented genotypes that were not found in isolates from CL individuals. Therefore, in the region studied, patients with ACL consist in a more homogeneous group of individuals than originally suspected, and are distinct from classical CL regarding treatment outcome, immune response and causative strain of *L. (V.) brazilensis*.

1735

MODELING ECO-BIO-SOCIAL DETERMINANTS FOR HOUSEHOLD INVASION OF SYLVATIC *TRIATOMA DIMIDIATA* IN NORTHERN BELIZE

Angela T. Caranci[1], John P. Grieco[2], Nicole L. Achee[3]

[1]Uniformed Services University, Kensington, MD, United States, [2]Uniformed Services University, Bethesda, MD, United States, [3]University of Notre Dame, Notre Dame, IN, United States

Initial reports have confirmed presence of *Triatoma dimidiata*, an important Chagas disease vector throughout northern Belize. To date, *T. dimidiata* remains the sole vector species reported from this Central American country, yet much of the disease transmission dynamics remain unclear. Here, we report updated infection rates of the vector population as well as infestation rates for villages in north and central Belize. In order to further characterize the epidemiological risk of human-vector contact, Over 225 households have been surveyed and characterized with respect to 30 key determinants related to the probability of household infestation by *T. dimidiata*. These key variables included: presence of domestic animals, distance of household to village periphery, and proximity of community light sources. The infestation behavior of *T. dimidiata* in Belize is confirmed to be distinct from what would classically be designated a domiciliated vector population. Risk factors reported here can be used to guide integrated control efforts to reduce infestation and limit human-vector contact.

1736

CHAGAS DISEASE IN MEXICO: SURVEILLANCE AND PERCEPTIONS OF BURDEN

Ellen Shelly[1], Rodolfo Acuna-Soto[2], Charles Sterling[1], Kacey Ernst[1], Heidi E. Brown[1]

[1]University of Arizona, Tucson, AZ, United States, [2]National Autonomous University of Mexico, Mexico City, Mexico

Chagas disease, a parasitic disease caused by *Trypanosoma cruzi*, disproportionately affects the poor throughout Latin America. We describe the spatial and temporal distribution of officially reported Chagas disease incidence and mortality in Mexico. The greatest burden appears to occur in southern states. Incidence rates and deaths were highest in adults (25-44 years and ≥45 years, respectively). We show increasing temporal trends for incidence (AR(2) p=0.002, 95% CI: 0.040-0.061) and mortality (MA(1) p < 0.0001, 95% CI: 0.012-0.021). While these results provide insight to the changing burden of Chagas in Mexico, under-reporting likely compromises our capacity to understand the epidemiology of this disease. The reported 500 new cases and 20 deaths in 2010 are in stark contrast to estimates of 69,000 new cases and 25,000 deaths per year from seroprevalence studies. As changes in Chagas surveillance improve our understanding of the full burden of this disease, it is likely that the reported and estimated

incidence will align more closely. This will facilitate understanding the epidemiology of this disease and result in more focused and successful control and prevention strategies.

1737

TRYPANOSOMA CRUZI AND OTHER TRYPANOSOMATIDS IN COMMONLY HUNTED WILD MAMMALS FROM REMOTE LOCATION OF THE PERUVIAN AMAZON

E. Angelo Morales[1], Pedro Mayor[2], Mark Bowler[3], Esar Aysanoa[1], Julio A. Ventocilla[1], Jocelyn Perez[4], G. Christian Baldeviano[1], Andres G. Lescano[1]

[1]U.S. Naval Medical Research Unit - 6, Lima, Peru, [2]Autonomous University of Barcelona, Barcelona, Spain, [3]San Diego Zoo Global, Iquitos, Peru, [4]University of Prince Edward Island, Charlottetown, PE, Canada

Chagas disease is generally transmitted by contact with the feces of triatomine bugs infected with *Trypanosoma cruzi*, but oral transmission has also been documented. We evaluated the prevalence of *T. cruzi* and other trypanosomatids in four orders of mammals hunted for subsistence to better understand the risk of infection associated with human consumption and contact. Blood samples from wild mammal species were collected on filter papers by subsistence hunters from a remote small community in Loreto, Peru, bordering Brazil. DNA was isolated from filter papers and amplified using a nested-PCR targeting the 24S alpha subunit rRNA gene. Primers D75/D76 and D71/D72 were used to amplify specific regions of trypanosomatids and *T. cruzi*, respectively. Comparisons of prevalence between orders were performed using Chi-Square and Fisher's exact tests. A total of 142 mammalian blood samples from four orders (10 species) were tested: Carnivora (n=34), Edentata (n=24), Artiodactyla (n=28) and Rodentia (n=56). The prevalence of *T. cruzi* in Carnivora (18%) was significantly higher (p=0.008) compared to other orders (0% - 4%). The prevalence of trypanosomatids ranged from 7% in Artiodactyla to 27% in Rodentia with no significant differences (p=0.180), possibly due to the small sample size. *Nasua nasua* (ring-tailed coati), *Dasypus novemcinctus* (nine-banded armadillo), *Agouti paca* (spotted paca) and *Tayassu tajacu* (collared peccary) accounted for 89% of the samples and all positive animals. Among these four species the prevalence of *T. cruzi* was 19%, 4%, 2% and 0%, respectively (p=0.016); and the prevalence of trypanosomatids ranged from 9% to 31%. The high prevalence of *T. cruzi* in Nasua nasua, a type of raccoon, suggests the importance of carnivores in sylvatic *T. cruzi* transmission. The multiple hunted species infected with trypanosomatids highlights the risk of human infection by consumption of improperly cooked meat.

1738

PREVALENCE OF TRYPANOSOMATIDS AND *TRYPANOSOMA CRUZI* IN WILD AND CAPTIVE NON-HUMAN PRIMATES FROM PERÚ

Esar Aysanoa[1], Pedro Mayor[2], A. Patricia Mendoza[3], E. Angelo Morales[1], Carlos M. Zariquiey[3], Jocelyn G. Perez[4], Mark Bowler[5], Julio A. Ventocilla[1], G. Christian Baldeviano[1], Andres G. Lescano[1]

[1]U.S. Naval Medical Research Unit - 6, Lima, Peru, [2]Autonomous University of Barcelona, Barcelona, Spain, [3]Wildlife Conservation Society, Lima, Peru, [4]University of Prince Edward Island, Charlottetown, PE, Canada, [5]San Diego Global Zoo, Iquitos, Peru

Non-human primates (NHPs) can be infected with *Trypanosoma cruzi*, the etiological agent of Chagas disease, and other trypanosomatids. Primates may act as reservoirs and close contact between humans (traders, owners, hunters, zookeepers, etc.) and NHPs is a potential risk of accidental infection. We compared the prevalence of *T. cruzi* and other trypanosomatids in wild and captive Peruvian NHPs to assess this risk. Blood samples were obtained from captive NHPs (n=192) at zoos, wildlife rescue centers, wet markets and households in six Peruvian cities, and wild NHP hunted for subsistence (n=126) in two remote communities in the Peruvian Amazon. Blood smears from 88 captive NHPs were stained with Giemsa and examined by microscopy. Samples were collected from 318 NHPs on filter paper, FTA cards or EDTA tubes and tested with a nested PCR protocol using primers for the 24S alpha subunit rRNA gene. Primers D75/D76 target the conserved flanking sequences of the D7 alpha domain in trypanosomatids, while primers D71/D72 target a region in the same domain that is specific to *T. cruzi*. PCR was used as gold standard to calculate the sensitivity and specificity of microscopy. Trypanosomatid and *T. cruzi* prevalences were compared using Chi2 and Fisher's exact tests. We studied captive NHPs from five families (14 species) and wild NHPs from three families (11 species). Wild NHPs had significantly higher prevalence of both trypanosomatids (56% vs 27%, p<0.001) and *T. cruzi* (9% vs 3%, p=0.034), compared to captive NHPs. Pitheciidae had the highest trypanosomatid prevalence (18/20, 90%) and Cebidae had the highest *T. cruzi* prevalence (14/116, 12%). Captive NHPs from wet markets (n=38) had very high trypanosomatid (53%) and *T. cruzi* (13%) prevalence. Compared to PCR, microscopy was 83% sensitive and 98% specific. *T. cruzi* and trypanosomatids are common in Peruvian NHPs and pose a risk to human and animal health that has not been properly studied. Although microscopy is poorly sensitive compared to PCR, it may still be useful for screening in the field.

1739

CHAGAS DISEASE, POVERTY AND BIODIVERSITY IN MEXICO

Alejandro Cruz-Reyes, Blanca Camargo-Camargo

Instituto de Biología, Universidad Nacional Autonoma De Mexico, Mexico City, Mexico

Since 1928 Chagas disease (ChD) has been studied in Mexico. Official data from the National Council for the Evaluation of the Politics for Social Development (Consejo Nacional de Evaluación de la Política de Desarrollo Social) [CONEVAL] in 2012 shows that poverty is 50% in Mexican population out of the 110 M people (2010 national census: National Institute of Statistic and Geographical Information) [INEGI], from this, 15% is under extreme poverty. In the Southern region, Mexico has high level of poverty and the highest biodiversity richness in the country, which is currently under serious ecological threating. There is evidence that reduced biodiversity affects the transmission of infectious diseases in humans and other animals. This situation matches perfectly in maps with the high prevalence of ChD in that region. The importance of this work is related to the role of multinational control initiatives against ChD. In some way ChD is a neglected disease in Mexico. This situation is also relevant giving the immigrant phenomenon between Mexico and the US. In this work we present data and geographical evidence that even the great number of Mexican scientists working on ChD, this zoonotic parasitic disease is still under estimation. Back in 2006 we published information about ChD in Mexico form our data base "CHAGMEX", now we are working on a new data base (2004-2014), and so far, the new bibliographic information shows that the number of human cases is increasing considering all clinical and epidemiological forms of the disease: vectorial transmission, blood transfusion, congenital transmission. From the 32 species of Triatominae identified in Mexico, more than 10 are reported with domestic habits. Also *Trypanosoma cruzi* is becoming quite common in domestic dogs, from urban and rural areas. We think that besides academic research, is urgent to implement vector control programs by each climatic region of Mexico, along with a wide epidemiological and socio-economical approaches of ChD in Mexico.

1740

MAPPING THE PREVALENCE AND CASE DETECTION RATES OF GAMBIENSE HUMAN AFRICAN TRYPANOSOMIASIS

Nick Golding[1], Simon I. Hay[1], Giuliano Cecchi[2], Massimo Paone[3], José R. Franco[4], Pere P. Simarro[4]

[1]University of Oxford, Oxford, United Kingdom, [2]Food and Agriculture Organization of the United Nations, Addis Ababa, Ethiopia, [3]Food and Agriculture Organization of the United Nations, Rome, Italy, [4]World Health Organization, Geneva, Switzerland

Recorded human cases of gambiense human African Trypanosomiasis (HAT) have undergone a remarkable decline over the past decade, prompting plans for elimination of the disease. Effective planning for this elimination programme will require accurate and reliable spatial information on the contemporary distribution of the disease. The Atlas of HAT, recently established by WHO and FAO, aims to map the locations of all known HAT cases. These data provide an unparalleled resource for spatial risk assessment. However, under-reporting of cases and spatial variation in reporting rates complicate their interpretation and reduce their utility for continental-scale planning. To overcome these issues, a collaboration between the Spatial Ecology and Epidemiology Group (Oxford), WHO and FAO is developing a spatial modelling framework to simultaneously map the prevalence of gambiense HAT cases and the probability of detection of cases through the passive reporting system. In order to construct this framework, a novel Bayesian spatio-temporal joint statistical model has been developed to integrate data from both active and passive case detection. The modelling framework will be outlined and preliminary results presented for the first time.

1741

THE HEALTH IMPACT OF VISCERAL LEISHMANIASIS AND HUMAN AFRICAN TRYPANOSOMIASIS WHEN REACHING THE 2020 WHO CONTROL AND ELIMINATION TARGETS

Epke le Rütte, Wilma Stolk, Nico Nagelkerke, Jan H. Richardus, Sake de Vlas

Erasmus MC, Rotterdam, Netherlands

Visceral leishmaniasis (VL) and human African trypanosomiasis (HAT) are neglected tropical diseases (NTDs). The London Declaration was established in 2012 to support the WHO control and elimination targets for ten NTDs by 2020. This initiative intends to globally generate a positive health and socioeconomic impact by decreasing and eliminating the disease burden caused by these NTDs. In our study the global health impact for VL and HAT is calculated for the ideal situation that the 2020 targets are met. The global burden of disease (GBD) study provides prevalence data for VL and HAT in 1990 and 2010. The 2020 targets, as formulated in the WHO Roadmap, provide the ideal situation: control for VL (100% detection and treatment at global level, and 1/10,000 new cases at subdistrict level per year on the Indian subcontinent) and elimination of HAT by 2030. Linear trends between 1990, 2010, 2020 and 2030 provide a simplification of the real situation, representing the number of remaining cases with disease, per country, age group and sex. Continuing the 1990 prevalence until 2030, corrected for demographic changes based on UNPOP data, serves as baseline situation without interventions. The difference between the baseline and the remaining cases results in the number of averted cases. The total number of averted years lived with disability (YLD) is calculated by multiplying the number of averted cases with the GBD disability weights. The total number of averted disability adjusted life years (DALYs) between 2010 and 2030 results in app. 140 and 100 million DALYs for VL and HAT, respectively. The DALYs are almost completely determined by the number of years of life lost (YLLs). The number of averted deaths over these two decades is 2.4 million and 1.7 million for VL and HAT, respectively. Although there have been many successful interventions for VL and HAT, it is important to emphasize the need for continuation and even increase of these efforts, especially when recognizing the sizeable health impact that can be gained when achieving the 2020 targets.

1742

ANTI-*LEISHMANIA* ANTIBODIES IN BLOOD DONORS FROM BRAZIL USING RECOMBINANT *L. INFANTUM* PROTEIN K39 ELISA

Maria Carmen A. Sanchez[1], Marina P. Dornelas[1], Silvano Wendel[2], Silvia M. Di Santi[1], Adeiana G. Lima[1], Beatriz J. Celeste[1], Hiro Goto[1]

[1]São Paulo University, São Paulo, Brazil, [2]Sirio Libanês Hospital, São Paulo, Brazil

In Brazil, visceral leishmaniasis (VL) caused by *Leishmania infantum* has a wide geographic distribution throughout the country with 3,894 reported cases in 2011, 47% in the Northeast region. Most of *L. infantum*-infected individuals (around 95%) are asymptomatic and may be undetected and accepted as blood donors in endemic areas. Since leishmaniasis can be transmitted through blood transfusion, this study aimed to investigate the presence of anti-*L. infantum* rK39 antibodies in blood samples from Brazil endemic areas. We used ELISA-rK39 (rK39 antigen kindly provided by Infectious Disease Research Institute, USA) that yielded 95.45% sensitivity, assaying 44 sera from parasitologically confirmed symptomatic VL patients with positive Direct Agglutination Test (DAT) and 100.0% specificity assaying 44 healthy endemic DAT negative control samples. The present study was carried out with 916 blood samples from Brazilian Northeastern states, Bahia (N=604) and Ceará (N=312). Anti-rK39 antibodies were detected in 26 out of 916 samples (2.8%): Bahia (2.8%) and Ceará (3.0%). The reactivity index (RI = absorbance/cut-off) varied from 1.010 to 6.756. Immunochromatographic rK39 test (ICT) applied to the 26 reactive samples showed one positive (from Ceará). Using *L. major*-like promastigote antigen (Lm), ELISA-Lm and indirect immunofluorescence test (IFT-Lm) detected respectively seven and one out of 26 positive samples. The sample showing RI of 6.756 in ELISA-rK39 was positive also in ELISA-Lm and ICT. Of note, the studied samples had been screened for Chagas' disease using antigen that cross-reacts with *Leishmania* and has been approved for transfusion; however, the present results showed that this assay using cross-reactive antigen missed those 26 samples with anti-*Leishmania* antibodies that likely result from an asymptomatic *L. infantum* infection. As in endemic areas, it is not easy to differentiate transfusion- or vector-mediated transmission; the occurrence of transmission by transfusion is probably underestimated and raises concerns on blood transfusion safety.

1743

THE RELATIONSHIP BETWEEN CLIMATIC AND OTHER ENVIRONMENTAL FACTORS AND ANNUAL FLUCTUATIONS IN INCIDENCE OF VISCERAL LEISHMANIASIS IN GEDAREF STATE, EASTERN SUDAN

Dia-Eldin A. Elnaiem[1], Alexandra Sweeney[2], Andrew Kruczkiewicz[2], Caitlin Reid[2], François Chappuis3[3], Koert Ritmeijer[4], Omran Osman[5], Madeleine Thomson[2], Ceccato Ceccato[2]

[1]University of Maryland Eastern Shore, Princess Anne, MD, United States, [2]International Research Institute for Climate and Society, Columbia University, Palisades, NY, United States, [3]MSF-Switzerland, Geneva, Switzerland, [4]MSF-Holland, Amsterdam, Netherlands, [5]University of Khartoum, Khartoum, Sudan

Visceral leishmaniasis (VL, kala azar), in Gedaref State, eastern Sudan, is caused by *Leishmania donovani*, which is transmitted by *Phlebotomus orientalis* sand flies. The endemicity of the disease in this region is characterized by marked annual fluctuations and occasional severe epidemics that claim the lives of many people. Since no significant interventions are undertaken against the disease in this region, the fall and rise in the incidence of VL in Gedaref state may be due to climate

variability, which characterizes the Sahelian region. Previous studies conducted by our groups and other research teams in Sudan and the Republic of South Sudan (RSS) showed marked variation in spatial distribution of kala azar that can be related to a number of environmental and socio-economic factors that may be acting together or independently to increase the vulnerability of specific populations to the disease. Our findings supported the previous notion that the vector and the disease are associated with *Acacia seyal - Balanites* woodland and chromic vertisol soils. We used this knowledge to produce a general kala azar risk map based on environmental prediction of the distribution of *P. orientalis*, the VL vector in Sudan and RSS. However, no attempt has yet been made to correlate annual incidence of kala azar with climatic factors and it is not known whether the flare up in disease incidence is associated with dry or wet years. In this study we analyzed VL records of MSF-Switzerland and MSF-Holland, form 1996-2004 and 2010-2012, in relation to a number of climatic and environmental variables, including temperature, humidity, rainfall and normalized difference vegetation index (NDVI). Our results indicated that the incidence of kala azar in this region is related to late onset of the rainy season. Results are discussed in relation to the epidemiology of the disease. Findings from the study may be used in the future to develop an Early Warning System and construct high resolution Geographical Information System (GIS) risk-maps for the disease.

1744

CO-STIMULATORY MOLECULES ARE INVOLVED IN ANERGY IN SYMPTOMATIC VISCERAL LEISHMANIASIS

João Firmino Rodrigues-Neto

Federal University of Rio Grande do Norte, Natal, Brazil

The proliferation and differentiation of naive T cells require signals provided by co-stimulatory molecules on antigen presenting cells (APCs), in addition to antigen-induced signals. In the absence of co-stimulatory signals, T cells become anergic. We hypothesized that co-stimulatory molecules might be involved in part in the reversible anergy observed during symptomatic visceral leishmaniasis. We analyzed the profile of co-stimulatory molecules in lymphocytes and in CD14+ monocytes in whole blood collected from subjects with symptomatic visceral leishmaniasis (sVL) and after their clinical recovery (rVL). An increase in CD8+ T cells expressing CTLA-4 after stimulation with soluble Leishmania antigens (SLA) was observed ($p<0.01$) in sVL. An increase in percentage of CTLA-4 in CD4+ and CD8+ in *ex vivo* condition was observed, when compared sVL versus rVL ($p<0.05$). No difference in lymphocytes expressing CD28 after SLA stimulation was observed in sVL or rVL, but rVL showed a high percentage of CD8+CD28+ in *ex vivo* condition when compared with sVL ($p<0.05$). An increase in the percentage of OX-40 in CD4+ T cells after SLA stimulation in sVL ($p<0.05$) was observed, as well as, an increase of ICOS in CD4+ and CD8+ T cells after SLA stimuli in sVL ($p<0.01$). Furthermore, a high percentage of ICOS in CD4+ in *ex vivo* condition was observed in sVL ($p<0.01$). There was no difference in CD40, CD86, CD80, ICOSL and HLA-DR in CD14+ monocytes after SLA stimulation in sVL or rVL. There wasn't also difference in the median fluorescence intensity (MFI) of CD40, CD80, ICOSL or HLA-DR after SLA stimulation in sVL or rVL observed in CD14+ monocytes. But, CD86 showed a high expression in rVL after SLA stimulation ($p<0.05$). These data support the role of co-stimulatory molecules in the reversible anergy observed during symptomatic VL and might indicate pathways to be explored for immunotherapy against leishmaniasis.

1745

DEFICIENCY OF PROLACTIN-INDUCIBLE PROTEIN LEADS TO IMPAIRED TH1 IMMUNE RESPONSE AND SUSCEPTIBILITY TO AN INTRACELLULAR PATHOGEN

Jintao Li[1], Dong Liu[1], Anne Blanchard[2], Ping Jia[1], Yvonne Myal[2], Jude Uzonna[1]

[1]Institute of Tropical Medicine, Third Military Medical University, Chongqing, China, [2]Department of Pathology and Deprtment of Physiology, Faculty of Medicine, University of Manitoba, Winnipeg, MB, Canada

The prolactin-inducible protein (PIP) is a secretory protein strategically located at several ports of pathogen entry into the body suggesting it might play a role in host defense. To date, no study has addressed the contributions of PIP in immunity against infectious agents. Here, we assessed the phenotype and responsiveness of immune cells from PIP KO mice to polyclonal T cell stimulators and model antigens *in vitro* and *in vivo*. We found comparable numbers of immune cells, (including T, B, natural killer and dendritic cells) in the primary and peripheral lymphoid organs of wild type and PIP KO mice. Further in-depth phenotypic analysis revealed that PIP KO mice had slightly but significantly lower numbers of $CD4^+$ T cells in their spleens and lymph nodes. $CD4^+$ T cells from PIP KO mice showed significantly decreased proliferation, IL-2 production and impaired Th1 differentiation *in vitro*. The impaired *in vitro* Th1 response was confirmed *in vivo* where $CD4^+$ T cells from OVA-immunized PIP KO mice showed significantly impaired proliferation and IFN-γ production following *in vitro* restimulation. Furthermore, PIP KO mice were highly susceptible to *Leishmania major* infection as evidence by inability to control lesion progression and parasite proliferation. This impaired resistant was associated with dramatic impairment in IFN-γ and nitric oxide production by splenic and draining lymph node cells from infected mice. Collectively, our findings implicate PIP as an important regulator of $CD4^+$ Th1 cell response, and play a critical role in resistance to intracellular pathogens.

1746

T CELL ACCUMULATION IN THE SPLEEN DURING CHRONIC PROGRESSIVE VISCERAL LEISHMANIASIS

Audrie A. Medina, Elvia Y. Osorio, Omar A. Saldarriaga, Peter C. Melby

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

Visceral leishmaniasis (VL), caused by the protozoan, *Leishmania donovani*, is a chronic systemic infection that contributes to half a million new cases each year. During progressive VL, there is profound expansion of immune cells in the spleen. Type 1 and type 2 cytokines are increased during VL in patients, but the T cell population in the spleen during VL has not been fully characterized. In the Syrian hamster model of progressive VL, which mimics human VL, we found a significant increase in expression of the T cell transcription factors Tbet, GATA3 and Foxp3 in spleen cells over the course of chronic infection. This suggests the presence of a mix of Th1, Th2 and Treg cells. In purified splenic CD4 T cells from 28 day infected hamsters we found an increase in Tbet ($p<0.001$) and GATA3 ($p=0.0087$) and Th1-associated chemokine receptors CXCR3 ($p<0.0001$) and CCR5 ($p<0.001$). There was no significant difference in Foxp3 or the Th2-associated chemokine receptor CCR4 in purified CD4 T cells from uninfected and infected animals. These data suggest both Th1 and Th2 cells are present in the spleen during chronic infection, although one would expect Th2 cells to prominently express CCR4. Notably, we also found a significant population of CD4 T cells that expressed both Tbet and GATA3. The increase in Th1 and Th2 cells in the spleen during chronic infection could be due to local proliferation or splenic recruitment by T cell attracting chemokines. We found an array of chemokines (CCL2, CCL4, CCL5, CCL17, CCL22) increased in the spleen of hamsters over a course of disease. This suggests that T cell attracting chemokines may be playing a role in T cell accumulation at the site of infection.

1747

EVALUATION OF THE EFFICACY OF ANTIGEN DELIVERY BY THE TRANSCUTANEOUS IMMUNIZATION ROUTE IN A MURINE MODEL OF CUTANEOUS LEISHMANIASIS

Ines Elakhal Naouar[1], Nancy Koles[1], Elaine Morrison[2], Mangala Rao[2], Carl Alving[2], Naomi Aronson[1]

[1]Uniformed Services University, Bethesda, MD, United States, [2]Walter Reed Army Institute of Research, Laboratory of Adjuvant and Antigen Research, Silver Spring, MD, United States

Transcutaneous immunization (TCI) is a novel attractive vaccination method which offers advantages over traditional vaccination routes, exploiting the abundance of antigen presenting cells in the skin. We provide the first report of Transcutaneous immunization (TCI) is a novel attractive vaccination method which offers advantages over traditional vaccination routes, exploiting the abundance of antigen presenting cells in the skin. We provide the first report of TCI induced immune responses to Leishmania antigens. Leishmania major soluble antigens (SLA) and Phlebotomus papatasi salivary gland homogenates (SGH) were delivered transcutaneously with cholera toxin (CT), a potent adjuvant for developing mucosal immunity. Sixty inbred BALB/c mice were immunized three times at weeks 0, 3 and 6 with the vaccine formulations (different doses of SLA or SGH, SLA+SGH). TCI was well tolerated. Two weeks after the last vaccine boost, we assessed humoral (IgG titer to antigens and CT) and cellular immune responses (IFN-γ ELIspot and cytokine levels from splenic cell culture). In contrast to SGH alone, we showed that transcutaneous immunization of mice with SLA resulted in high titers of anti-SLA IgG that increased when SLA was combined with SGH antigen. Immunization was also associated with high anti-CT IgG titers. A Th1-type immune response was demonstrated with high levels of IFN-γ production and lower levels of IL-10 resulting in a significantly higher IFN-γ/IL-10 ratio compared to the control groups. A high frequency of IFN-γ secreting cells was also seen in groups of mice immunized with SLA. Altogether, these data are consistent with reported protective immune responses and indicate the strong potential of our TCI strategy to protect against Leishmania major infection, with the combined antigen SLA and SGH showing the strongest responses. Experiments using the same regimen of immunizations followed by parasite challenge are in progress. Results of lesion evolution and parasite load along with immune responses pre and post challenge will be presented.

1748

MECHANISMS OF DISEASE-PROMOTING MACROPHAGE PROLIFERATION IN VISCERAL LEISHMANIASIS

E. Yaneth Osorio[1], Audrie A. Medina[1], Omar A. Saldarriaga[1], Bruno L. Travi[1], Alda M. da-Cruz[2], Peter C. Melby[1]

[1]University of Texas Medical Branch, Galveston, TX, United States, [2]FIOCRUZ, Rio de Janeiro, Brazil

Macrophages have classically been viewed as mature non-proliferative cells, which originate from bone marrow monocytes released to peripheral blood to infiltrate tissues in response to injury. However, recently it was discovered that macrophages can be locally self-maintained without major contribution of infiltrating monocytes, and that M2 macrophages are able to proliferate locally within a Th2 environment. The mechanism(s) that drive this proliferation have not been defined. In a model of chronic progressive visceral leishmaniasis, we found huge expansion of disease promoting macrophages in the spleen, with signs of Th2-amplified M2 activation, increased STAT-6 activation, and pathological arginase expression. We discovered that growth factors IGF-I and FGF-2 were key contributors to both STAT-6 activation and arginase expression in *L. donovani* infected macrophages, and inhibition of growth factor signaling blocked arginase expression and parasite replication. Since that these growth factors also drive cellular proliferation and differentiation, and arginase contributes to the cell growth through polyamine production, we explored the possibility that these factors had a role in macrophage expansion in visceral leishmaniasis. *L. donovani* infection of bone marrow and splenic macrophages resulted in increased cell number, DNA synthesis (BrDu incorporation) and mitosis (ki-67 antigen expression). The combination of growth factor FGF-2 or IGF-I with IL-4 significantly increased macrophage proliferation, suggesting that they interact to control the cell cycle. Inhibition of FGFR, IGFR and PI3K significantly reduced mitosis indicating that growth factor signaling through PI3K was a major contributor to macrophage proliferation. The local amplification of macrophages in response to chronic infection through the expression of type 2 cytokines and growth factors may have broad significance to other chronic infectious diseases.

1749

FACTORS CONTRIBUTING TO TISSUE DAMAGE IN HUMAN CUTANEOUS LEISHMANIASIS

Sara T. Passos, Taís Menezes, Patrícia Cisneiros, Paulo R. Machado, Juliana Almeida, Viviane Magalhães, Viviane Magalhães, Lucas P. Carvalho, Edgar M. Carvalho

Federal University of Bahia, Salvador, Brazil

Human cutaneous leishmaniasis (CL), due to *Leishmania braziliensis* infection, is characterized by intense immune mediated tissue inflammation and skin ulceration. For many years immunological studies in CL patients have focused on the evaluation of host response mainly using peripheral blood cells assays. In this context, high levels of IFN-gamma and TNF and low levels of regulatory cytokine, IL-10, is observed in cultures of peripheral blood mononuclear cells stimulated with soluble *Leishmania* antigen. Histopathology studies show mononuclear cells infiltration and low number of parasites. Our hypothesis is that the inflammatory environment helps to control parasitemia but mediates tissue destruction. In the present study we evaluated cytokine and chemokine profile at lesion site. Cells from CL lesion produced high levels of pro-inflammatory cytokines, TNF, IL-6 and IL-1b in absence of stimuli. To determine the contribution of skin epithelial cells to the production of these cytokines we cultured epidermis and dermis separately. TNF was only produced by cells composing the dermis, while IL-6 and IL-1b were produced by dermis and epithelial cells from epidermis. High levels of CCL2, a chemokine that recruits mononuclear phagocytes, and CXCL9 and CXCL10, involved in lymphocyte recruitment, were also observed. Metalloproneinase-9 (MMP-9) is a zinc-dependent enzyme that degrades collagen type 4 (present in basal membrane) and has been associated with tissue damage in skin inflammatory diseases. We found increased production of MMP-9 in CL lesion when compared to healthy skin. TNF is known to induce MMP-9 production. To determine the role of TNF in MMP-9 production in CL, we cultured CL cells in presence of monoclonal antibodyes anti-TNF. Blockage of TNF decreased MMP-9 production in CL. Ou study contributes to the understanding of immunopathology in CL and revele possible targets for immunotherapy.

1750

IMMUNOGENICITY AND PROTECTIVE EFFICACY OF PVAX-NH36 AS A DNA VACCINE AGAINST CUTANEOUS LEISHMANIASIS IN A CANINE MODEL

Vladimir Cruz-Chan[1], Amaru Aguilar-Cetina[1], Liliana Villanueva-Lizama[1], Pedro Martinez-Vega[1], Maria Jesus Ramirez-Sierra[1], Miguel Rosado-Vallado[1], Leonardo Guillermo-Cordero[1], Jaime Ortega-Lopez[2], Eric Dumonteil[1]

[1]Universidad Autonoma de Yucatan, Merida, Yucatan, Mexico, [2]CINVESTAV, Mexico, D.F., Mexico

Leishmaniasis is a major tropical disease affecting 2 million people annually, against which there is no effective treatment. Vaccine development for humans requires preclinical testing in animal models including canines. One of the best vaccine candidates is NH36, a *Leishmania donovani* 36 kDa protein. As DNA vaccine NH36 provides better protection than the recombinant protein or purified fucose-manose

ligand. In this study, we evaluated the immunogenicity and protection of pVAX-NH36 for the prevention and therapy of *L. mexicana* infection in dogs. We first established a model of infection in Beagle dogs. Then, Beagles received 3 doses of 250 microg of pVAX-NH36 as prophylaxis or therapy, while dogs from control group received saline solution. *L. mexicana* promastigotes were used for infection via intradermal. Immune response was evaluated measuring antibodies, IFN and IL-10 production, and DTH. Ulcer diameters and parasite burden were evaluated to assess protection. Canines receiving pVAX-NH36 showed higher IgG levels against NH36 in comparison with the control group. High IFN and low IL-10 levels were produced by PBMC stimulated with NH36 from vaccinated canines. In addition, only vaccinated animals were DTH positive against recombinant NH36. Finally, some protection was observed based on skin parasite burden as two vaccinated animals showed negative results by qPCR. In conclusion, pVAX-NH36 is safe and immunogenic in dogs, and can confer some protection against *L. mexicana*.

1751

LEISHMANIA SPECIFIC CD4 T CELLS RELEASE IFNΓ THAT LIMITS PARASITE REPLICATION IN PATIENTS WITH VISCERAL LEISHMANIASIS

Om Prakash Singh[1], Rajiv Kumar[1], Neetu Singh[1], Shalini Gautam[1], Kamlesh Gidwani[1], Madhukar Rai[1], David Sacks[2], Shyam Sundar[1], Susanne Nylen[3]

[1]Institute of Medical Sciences, Banaras Hindu University, Varanasi, India, [2]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [3]Karolinska Institutet, Department of Microbiology Tumor and Cell Biology, Stockholm, Sweden

Visceral leishmaniasis (VL) is associated with increased circulating levels of multiple pro-inflammatory cytokines and chemokines, including IL-12, IFNγ, and TNF-alpha, and elevated expression of IFNγ mRNA in lesional tissue such as the spleen and bone marrow. However, an immunological feature of VL patients is that their peripheral blood mononuclear cells (PBMCs) typically fail to respond to stimulation with leishmanial antigen. Unexpectedly, it was recently shown that Leishmania specific IFNγ, can readily be detected when a whole blood stimulation assay (WBA) is used. We sought to define the conditions that permit whole blood cells to respond to antigen stimulation, and clarify the biological role of the IFNγ found to be released by cells from VL patients. CD4+ T cells were found to be crucial for and the main source of the IFNγ production in Leishmania stimulated whole blood (WB) cultures. Complement, antibodies and red blood cells present in whole blood do not play a significant role in the IFNγ response. The IFNγ production was reduced by blockade of human leukocyte antigen (HLA)-DR, indicating that the response to leishmanial antigens observed in WB of active VL patients is a classical HLA- T cell receptor (TCR) driven reaction. Most importantly, blockade of IFNγ in ex-vivo splenic aspirate cultures demonstrated that despite the progressive nature of their disease, the endogenous IFNγ produced in patients with active VL serves to limit parasite growth.

1752

ISOLATION AND INITIAL CHARACTERIZATION OF *TRYPANOSOMA CRUZI* ISOLATES FROM A POPULATION OF CYNOMOLGUS MACAQUES NATURALLY INFECTED IN THE UNITED STATES

Phil Yao

University of Georgia, Athens, GA, United States

The protozoan parasite *Trypanosoma cruzi*, causative agent of Chagas disease, is capable of infecting not only humans, but also virtually any mammalian species. Non-human primates housed in outdoor facilities in the southern United States are known to acquire *T. cruzi* infection, as is the case for a group of 64 cynomolgus macaques from Texas who are seropositive for *T. cruzi* infection. This group provided a unique opportunity to investigate natural *T. cruzi* infection in an entire population from a locale. Similar to endemic human infection, infection in these macaques is thought to have been acquired early in life (age range at infection 1-15 years) and to persist chronically with an average infection time of 6.4 years. The *T. cruzi* isolates were obtained from hemocultures of 43 out of 64 (67%) macaques. An additional 8 hemocultures were positive for *T. cruzi* DNA by PCR, but failed to yield a culturable line, resulting in an overall hemoculture detection rate of 80%. This figure is comparable to the frequency of detection of infection by serial PCR (up to 3 samples) of whole blood (81%). Furthermore, parasite isolates were obtained by hemoculture from 8 of the 12 PCR-negative animals. The combination of whole blood PCR and hemoculture +/- PCR confirmed active infection in 94% of the seropositive animals. Genotyping of hemoculture-isolated *T. cruzi* revealed the presence of two lineages: TcI and TcIV, which are the most common lineages identified by previous studies in infections originating in North America. To date, all tested hemoculture-isolated *T. cruzi* could be converted to metacyclic trypomastigotes and were orally infective in C57Bl/6 and IFN-gamma knockout mice with the TcIV lineage isolates exhibiting less virulence in both mouse models. All isolates appeared to establish chronic infection in mice and to induce immune responses consistent with those observed in infections by long-maintained laboratory-adapted strains of *T. cruzi*. Future studies will assess the susceptibility of these fresh isolates to clearance by treatment with benznidazole.

1753

IMMUNOREGULATORY NETWORKS AND IMMUNOPATHOGENIC PATHWAYS IN VISCERAL LEISHMANIASIS

Omar A. Saldarriaga, Fanping Kong, Heidi Spratt, Bruce A. Luxon, Elvia Y. Osorio, Bruno L. Travi, Peter C. Melby

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

Visceral Leishmaniasis (VL) caused by *Leishmania donovani*, is an important problem in tropical and subtropical areas of the world. VL is characterized by a progressive increase in visceral parasite burden, cachexia, splenomegaly, pancytopenia and ultimately death. We have studied the pathogenesis of VL in Syrian hamsters *(Mesocricetus auratus*) since it closely mimics active human disease. We demonstrated previously that the simultaneous expression of Th1 (IFN-γ) and Th2 (IL-4) cytokines, parasite activation of STAT6, and decreased production of nitric oxide are associated with susceptibility to the infection. To fill the gaps in our understanding of the pathogenesis of VL we examined global changes in gene expression in spleen tissue and splenic (adherent) macrophages. We used a novel approach of deep sequencing (RNAseq) coupled with *de novo* assembly of full-length transcripts because the Syrian hamster does not have an annotated reference genome. Differentially expressed transcripts in *L. donovani* infected (28 days) vs. uninfected hamsters were determined by alignment back to the *de novo* constructed transcriptome and cross-species BLAST, after the removal of contaminating parasite sequences. Transcriptome analysis confirmed that adherent cells were enriched for expression of macrophage (CD14, CD64 and Mertk) but not T cells markers (CD4, Gata3 and Tbet). Differentially expressed genes analyzed with IPA software revealed a number of highly enriched canonical pathways in spleen and splenic macrophages, including hepatic fibrosis, pathogenesis of multiple sclerosis, atherosclerosis signaling, communication between innate and adaptive immune cells, and the glucocorticoid receptor signaling. Notably, within the differentially expressed transcripts we identified mixed expression of genes associated with classical (M1) and alternative (M2) activation of macrophages that was confirmed by qPCR. This approach provides a valuable tool to overcome the obstacles of working with a non-model organism without a reference genome. With it we can begin to understand the complex immunopathogenic mechanisms at the site of visceral infection.

1754

ENHANCEMENT OF MURINE VISCERAL LEISHMANIASIS DUE TO CROSS REACTIVE *LEISHMANIA MAJOR* ANTIBODIES

Heidi Anderson, Blaise Dondji, **Gabrielle A. Stryker**

Central Washington University, Ellensburg, WA, United States

Leishmaniasis is a global disease found in regions with compatible temperatures for the phlebotomine sandfly vector to survive and lacking rigorous vector control programs. An estimated 1.3 million new cases and 20,000 - 30,000 deaths occur annually due to this parasitic protozoan. More than twenty different species of *Leishmania* infect humans with multiple species occurring in the same geographic areas. Symptoms range from a minor cutaneous lesion at the bite site due to dermotropic species such as *L. major*, to life threatening disseminated disease with multiple organ involvement, caused by viscerotropic species such as *L. infantum*. We have previously shown susceptible BALB/c mice infected with a low/self-healing dose of cutaneous *L. major* and challenged with *L. infantum*, develop a markedly worsened disease with higher parasite burden, relative to naïve mice. There was little notable difference in the cytokine profiles between *L. major* exposed and naïve mice in response to *L. infantum*. Cross-reactive antibodies were seen in both groups of *L. infantum* infected mice regardless of their immune history. Opsonizing antibodies have been shown to lead to increased disease in visceral leishmaniasis. The present studies focus on exploring the role cross-reactive antibodies may play in exacerbation of visceral disease seen in mice previously exposed to *L. major*. Mice receiving passively transferred serum from *L. major* infected mice, 48 hours prior to challenge with *L. infantum,* developed equivalent organ parasitemia to age/*L. major*-infected matched control mice. Naïve mice inoculated with control serum did not suffer any disease enhancement with *L. infantum*. We speculate that cross-reactive antibodies are augmenting visceral disease in mice with immunological memory to *L. major*. While *L. major* is known to produce long lasting immunological memory and protect against recurrent cutaneous disease, antibody enhancement due to inter-*Leishmania* infection may enhance disease in regions with multiple circulating *Leishmania*-species and suggests leishmanization might be riskier than previously thought.

1755

HUMAN SEROPREVALENCE OF LEPTOSPIROSIS AND RICKETTSIOSIS IN FOUR REGIONS OF PERU

Gabriela Salmon-Mulanovich[1], Mark Simons[1], Maria E. Silva[1], Bruno M. Ghersi[2], Matthew R. Kasper[3], Hugo R. Razuri[4], Daniel G. Bausch[5], Allen L. Richards[6]

[1]U.S. Naval Medical Research Unit - 6, Callao, Peru, [2]Tulane University, New Orleans, LA, United States, [3]Armed Forces Health Surveillance Center, Silver Spring, MD, United States, [4]McGill University Health Centre, Montreal, QC, Canada, [5]Tulane School of Public Health and Tropical Medicine, New Orleans, LA, United States, [6]Naval Medical Research Center, Silver Spring, MD, United States

Rickettsia and *Leptospira* are obligate intracellular bacteria with global distributions and a wide variety of animal hosts. Two disease-causing groups of *Rickettsia* are noted: the spotted fever group *Rickettsia* (SFGR) and the typhus fever group *Rickettsia* (TFGR). We explored the prevalence of exposure and risk factors for human *Rickettsia* infection in four ecologically distinct regions of Peru (Lima, Cusco, Puerto Maldonado and Tumbes) and for *Leptospira* in Puerto Maldonado and Tumbes. In January 2012 we randomly collected 2165 serum samples from participants in a surveillance cohort for respiratory disease in the aforementioned sites and tested them for IgG by ELISA (*Rickettsia*) and microscopic agglutination test (*Leptospira*). Overall antibody prevalence across the four sites was 10.6% for SFGR (ranging from 6.2-14.0%, with the highest prevalence in Tumbes) and 3.3% for TFGR (ranging from 2.6-6.4%, with highest prevalence in Puerto Maldonado). Factors associated with positive IgG for SFGR on multiple logistic regression analysis were male sex (OR 2.2, 95% CI 1.5-3.3), increasing age (OR 1.02, 95% CI 1.01-1.04 per year), contact with backyard birds (OR 2.1, 95% CI 1.4-3.0), and working in agriculture or with livestock (OR 4.3, 95% CI 1.7-10.8). However, exposure to any kind of animal within the household decreased the odds ratio by half (OR 0.50, 95% CI 0.31-0.81), perhaps indicating that arthropod vectors on birds preferred non-human hosts when they were present, thus diminishing exposure to humans. Age was the only variable associated with antibody positivity to TFGR (OR 1.03, 95% CI 1.02-1.05). The antibody prevalence to *Leptospira* was 11.3% in Puerto Maldonado and 5.8% in Tumbes, with a borderline association with keeping animals in the household (OR 2.5, 95% CI 1.0-6.2). Exposure to *Rickettsia*, especially SFGR, and *Leptospira* appears to be frequent in Peru. We plan now to perform testing in domestic animals in some of these sites to determine the specific reservoirs and vectors for these agents and to obtain pathogen isolates for identification of the specific species.

1756

PROXIMITY TO PIG POPULATIONS AS A KEY RISK FACTOR FOR JAPANESE ENCEPHALITIS DISEASE; RESULTS OF A FIVE-YEAR SURVEILLANCE STUDY FROM NORTHWESTERN BANGLADESH

Henrik Salje[1], Salah Uddin Khan[2], Abu Hannan[3], M. Atiqul Islam[4], A. A. Mamun Bhuyan[4], M. Ariful Islam[4], M. Ziaur Rahman[4], Mahmadur Rahman[5], Nazmun Nahar[4], M. Jahangir Hossain[4], Stephen P. Luby[6], Emily S. Gurley[4]

[1]Johns Hopkins School of Public Health, Baltimore, MD, United States, [2]University of Florida, Gainesville, FL, United States, [3]Fisheries Ministry, Dhaka, Bangladesh, [4]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [5]IEDCR, Dhaka, Bangladesh, [6]Stanford University, Stanford, CA, United States

Japanese encephalitis (JE) is a mosquito-borne virus that causes severe disease in humans with 10-20% case fatality. It is the commonest cause of encephalitis in Bangladesh. Humans are dead-end hosts. Pigs, by contrast, are viral amplifying hosts, so the distribution of pig populations may be particularly important for human disease risk. Previous studies have shown that the highest incidence of human JE infections occur in northwestern Bangladesh, where JE is also endemic among pigs. The objective of this study was to explore proximity to pigs as a risk factor for human JE disease in Bangladesh. We first geocoded the locations of residence for all JE patients identified through hospital-based surveillance in Naogaon, Chapainawabganj, and Rajshahi districts in northwestern Bangladesh between 2007 and 2011. Next, we used data from a 2009 pig census in these areas to map all pig raising households. To explore the impact of proximity to pigs as a risk factor for JE disease we compared the odds of a human JE case living within a set distance of a pig-raising household to that of a randomly selected control population. We identified 81 human JE cases from throughout the region, with a mean age of 32 years (range: 0 - 75 years); 11% died. Disease patterns were highly seasonal with 90% of cases occurring between the months of August and November. Humans infected with JE were 2.7 times more likely to live within 500m of a pig-owning household compared to controls (95% confidence interval [CI] 1.3 - 4.6) and 1.7 times more likely to live within 5km (95% CI: 1.0 - 3.7). Results from this analysis suggest that proximity to pig populations is an important risk factor for human JE disease in northwestern Bangladesh. JE vaccination is not currently included in the Bangladesh immunization program; therefore, interventions to reduce infections among pigs could be an important strategy for reducing human risk in these areas and should be explored.

1757

ECOLOGICAL NICHE MODELING FOR SYLVATIC RABIES TRANSMITTED BY *DESMODUS ROTUNDUS* IN PERU

M. Claudia Guezala Villavicencio[1], William D. Valerrama[2], Gabriela Salmon-Mulanovich[1], Daniel G. Bausch[3]

[1]U.S. Naval Medical Research Unit - 6, Callao, Peru, [2]Servicio Nacional de Sanidad Agraria, La Molina, Peru, [3]Tulane School of Public Health and Tropical Medicine, New Orleans, LA, United States

Rabies is a viral infection endemic in many parts of the world that causes severe and usually fatal encephalitis. In Latin America, a sylvatic cycle exists in which rabies virus is maintained in several species of wild animals, especially the hematophagous bat *Desmodus rotundus*. Peru and Brazil report the highest number sylvatic rabies cases, mainly transmitted by bats. Vaccination of humans and livestock is an effective prevention method, but vaccination is not usually undertaken until a human exposure our outbreak in livestock occurs. The ecologic and topographic factors underlying the distribution of rabies vectors are not well understood. Specifically for sylvatic rabies, there is little data on the factors influencing the distribution of hematophagous bats. Such information would help define areas and populations at risk for rabies and inform effective prevention campaigns. We therefore modeled the potential geographic distribution of *D. rotundus* using the ecological niche modeling algorithm MaxEnt. Incorporating climatic, environmental and anthropogenic factors that may relate to the geographic distribution of *D. rotundus* and rabies-infected farm animals, we developed a risk map for bat-associated rabies transmission in Peru. *D. rotundus* occurrence was found to be associated with the colder, drier months of the year. In addition, land classification data show that bats prefer firmer, non-flooding low lands. Variables associated with occurrence of animal rabies included livestock population density, mean diurnal temperature range, and precipitation in the drier months of the year. This study offers a first glimpse of the environmental and bioclimatic factors associated with the distribution of hematophagous bats and animal rabies using novel techniques that extract the maximum information and offer robust results from the little data available.

1758

THE PROBLEM OF LEPTOSPIROSIS IN AFRICA: REVEALING A NEGLECTED 'ONE HEALTH' CHALLENGE THROUGH A SYSTEMATIC REVIEW

Kathryn J. Allan[1], Holly M. Biggs[2], Jo E. Halliday[1], Rudovick R. Kazwala[3], Venance P. Maro[4], Sarah Cleaveland[1], John A. Crump[2]

[1]University of Glasgow, Glasgow, United Kingdom, [2]Duke University Medical Center, Durham, NC, United States, [3]Sokoine University of Agriculture, Morogoro, United Republic of Tanzania, [4]Kilimanjaro Christian Medical Centre, Moshi, United Republic of Tanzania

Recent evidence from Tanzania indicates that leptospirosis is an important cause of non-malarial febrile disease. However, relatively little is known about the incidence and geographic distribution of leptospirosis in Africa as well as the diversity of infecting *Leptospira* spp. in human and animal populations. To examine current knowledge, we performed a systematic review of acute human leptospirosis and confirmed *Leptospira* infection in animals. We searched eight international and regional scientific databases using the terms '*Leptospira*' OR 'leptospirosis' AND 'Africa' for articles published between 1930 and June 2013. Of 630 unique articles identified and reviewed against predetermined inclusion and exclusion criteria, 89 (14.1%) were considered eligible. Eligible articles described human and animal *Leptospira* spp. infection in 26 (44.8%) of 58 countries included in the UN continental definition of Africa. Prevalence of acute leptospirosis in hospital-based cohort studies of patients with non-malarial febrile illness ranged from 2.3% (n=43) to 47.5% (n=59). Estimates of annual human leptospirosis incidence ranged from 4.1 to 101 cases per 100,000 based on surveillance studies of island populations. *Leptospira* spp. infection was also reported in a wide range of animal hosts. 11 out of 15 human-infecting *Leptospira* serogroups were isolated from one or more animal host species in Africa. For several important human-infecting serogroups, multiple animal host species were identified. *L. borgpetersenii, L. interrogans* and *L. kirschneri* were the predominant genetic species reported in human and animal populations across Africa, although some local variation was observed. In conclusion, this systematic review highlights the importance of acute leptospirosis in febrile patients in Africa and reveals many areas of uncertainty that remain in our understanding of this complex, multi-host disease. A 'One Health' approach is advocated to integrate human and animal studies in future work, and to explore local and regional variation in leptospirosis epidemiology in Africa.

1759

CHARACTERIZING EXPOSURE TO BATS AND BAT GUANO AMONG MEN, WOMEN AND CHILDREN IN LAO PDR TO INFORM INTERVENTIONS FOR REDUCING THE RISK OF ZOONOTIC DISEASE

Laura Seckel[1], Khounkham Xaymounvong[2], Sara Woldehanna[1], Susan Zimicki[1]

[1]FHI 360, Washington, DC, United States, [2]FHI 360, Vientiane, Lao People's Democratic Republic

Bats host more viruses per species than other mammals, and are reservoirs of numerous pathogens that pose significant risk to humans. Several attention-grabbing emerging diseases, such as SARS, Ebola, Nipah and MERS-CoV have been linked to bats. Transmission routes for these, and other zoonotic diseases, are extremely varied, and bats are thought to transmit diseases through urine, feces, saliva or via intermediate hosts. To inform development of interventions to prevent the spread of disease from bats to humans, human exposure to bats and their excrement must be better understood. In Lao PDR, a country of great biodiversity, people are regularly exposed to bats in a variety of ways, including hunting, consumption, and the collection and use of guano. In addition, both humans and domestic animals are often exposed to bats and their excreta through environmental exposure. We used rapid appraisal and participatory research methods to characterize bat exposure among men, women and children in four sites in Vientiane and Bolikhamxay provinces, Lao PDR. We will report findings about human interactions with bats/excreta, environmental exposure of humans and domestic animals to bats/excreta, and the seasonality of exposure, mediated by gender, location, ethnicity (Lao-Tai, Hmong, Kammu), age, and occupation and will discuss possible preventive interventions.

1760

RISK FACTORS FOR HUMAN EXPOSURE TO WILDLIFE ZOONOSES IN NIGERIAN HUNTING COMMUNITIES

Sagan Friant, Tony Goldberg, Sarah Paige

University of Wisconsin Madison, Madison, WI, United States

Bushmeat hunting clearly increases the risk of zoonotic pathogen transmission. However, limited information exists on the nature and frequency of contact with wildlife in communities that practice bushmeat hunting, especially with respect to socioeconomic drivers of hunting behavior. We surveyed 188 hunters and 139 non-hunters in five rural communities in Cross River State, Nigeria. Responses were used to: 1) quantify contact rates with wildlife, 2) identify socioeconomic factors predisposing individuals to hunt, and 3) identify specific hunting behaviors that increase frequency of contact with wildlife. Among hunters, 95% hunted rodents, 91% ungulates, 90% carnivores, 78% primates and 35% bats. Hunters used traps (75%), guns (71%), machetes (71%), and dogs (18%) to hunt animals both day (78%) and night (69%). We constructed generalized linear mixed models to examine socioeconomic predictors of individual hunting behavior and frequency of contact with wildlife, especially primates. We found that lower education level ($<.01$), having a father who hunts ($p<.0001$), and larger household sizes ($p<.05$) were all associated with becoming a hunter. Among hunters, high rates of wildlife contact were associated with high hunting frequency ($p<.05$), hunting

both night and day (p<.05) and with a gun (p<.05); while sleeping in the forest (p<.0001), hunting night and day (p<.0001) and with a dog (p<.05) were associated specifically with high rates of primate contact. Results demonstrate that hunters have risky contact with a diversity of wildlife, and that the decision to become a hunter is deeply rooted in family history and modified by economic necessity. Improved education, reduced family sizes, and alternative livelihoods may reduce the risk of zoonotic disease exposure in rural hunting communities in Nigeria. Public health programs aimed at reducing zoonotic transmission of wildlife pathogens in such settings will be most efficient when they target root socioeconomic drivers that lead to hunting behavior and risky wildlife contact.

1761

ENVIRONMENTAL RESERVOIRS OF ANTIBIOTIC RESISTANCE ASSOCIATED WITH SMALL SCALE POULTRY FARMING IN NORTHWESTERN ECUADOR

Nikolay P. Braykov[1], Joseph Eisenberg[2], Marissa Grossman[1], Lixin Zhang[2], William Cevallos[3], Karla Vasquez[4], Diana Muñoz[4], Carl F. Marrs[2], Betsy Foxman[2], Gabriel Trueba[4], Karen Levy[1]

[1]Emory University, Atlanta, GA, United States, [2]University of Michigan School of Public Health, Ann Arbor, MI, United States, [3]Universidad Central del Ecuador, Quito, Ecuador, [4]Universidad San Francisco de Quito, Quito, Ecuador

Non-therapeutic use of antibiotics in agriculture poses a threat to human health by contributing to an environmental reservoir of antibiotic-resistant (AR) bacteria. Small-scale "backyard" broiler chicken production involving use of antibiotics for feed promotion is becoming increasingly common in developing countries. We use E. coli antibiotic susceptibility profiles to assess the potential for transmission of AR from broiler chickens to the surrounding environment (water, soil, surface) in the context of small-scale poultry farming in Northwestern Ecuador. Field work spanned 190 households in 17 villages visited between 08/2010-08/2012. We collected a total of 529 samples from drinking water, soil and food preparation surfaces, and surveyed water, sanitation and antibiotic use practices. From a subset of 91 households involved in broiler production, we collected 131 cloacal samples from production chickens and 66 soil and surface samples from chicken coops. In addition, 153 non-production ("free-range") birds were sampled across all villages, and 54 water samples from local rivers were collected. Up to five E. coli isolates from each sample were tested against 12 antibiotics using disc diffusion. Zones of inhibition and their categorical interpretations were compared using mixed-effect models. AR was more common in broilers than free-range chickens for every antibiotic tested (p<0.01), particularly tetracycline (76.8% vs 33.1%), sulfisoxazole (66.9% vs 19.2%) and streptomycin (61.1% vs 26.7%). A pattern of AR to gentamicin, fluoroquinolones and beta-lactams that was unique to broilers was also found in coop surfaces and soils, but not in household samples. The prevalence of this phenotype declined with bird age, implying importation form hatcheries outside the study system. AR was more common in coop than household samples. Farming and non-farming households showed no difference in AR profiles. These results suggest broiler chickens carry AR and pass it to their immediate environment. However, transmission to households may operate at different scales.

1762

FACTORS ASSOCIATED WITH *GIARDIA* IN HUMANS AND ANIMALS RESIDING IN RURAL AND URBAN AREAS OF COASTAL ODISHA, INDIA AND ENVIRONMENTAL LOADING ESTIMATES FROM ANIMALS

Miles Daniels[1], Woutrina Miller[1], Arpit Shivastava[2], Priyadarshi Sahu[2], Mitsunori Odagiri[1], **Marion W. Jenkins**[1]

[1]University of California Davis, Davis, CA, United States, [2]Kalinga Institute of Industrial Technology, Bhubaneswar, India

Globally, *Giardia lamblia*, is one of the most commonly detected intestinal parasites and is particularly relevant in developing countries such as India, where inadequate sanitary conditions, high population densities, and frequent contact with animals capable of carrying zoonotic pathogens can exist in both urban slums and rural areas. We present a study to estimate and compare the prevalence of *Giardia* infection in rural and urban settings for both human and animal populations residing in coastal Odisha, India, evaluate if gender or age is associated with *Giardia* infection in humans, and estimate fecal loading rates of *Giardia* cysts from animal host populations residing in the study area. From April to May of 2012, human fecal samples from 85 diarrhea patients presenting at three diarrhea wards and 111 pooled animal fecal samples across seven host species (cattle, buffalo, goat, sheep, chicken, cat, and dog) were collected across urban and rural residential areas served by the wards. Samples were screened and fluorescent microscopy used to enumerate *Giardia* cysts and a subset of dog and human samples analyzed by molecular methods to identify isolate genotypes. *Giardia* cysts were detected in 12% of tested diarrhea patients, while 32% of pooled animal samples were positive. No evidence for difference in the presence of *Giardia* cysts among humans was observed between urban and rural settings, gender, or age groups (<5 years, 5-59 years, >59 years). There was substantial support for a location effect on *Giardia* shedding among animals, with rural animals shedding higher numbers of parasites. Of the seven animal host groups screened, dogs and cattle, both reported to shed zoonotic genotypes of *Giardia* in India, shed decisively more *Giardia* cysts per gram of feces, as much as 2-3 orders of magnitude greater than other animal types (adjusting for location). Molecular characterization of isolates identified host specific Assemblages in dog samples and a possible zoonotic Assemblage in a human sample. Using current animal populations and observed *Giardia* shedding rates, cattle were estimated to contribute >99% of *Giardia* animal cysts into the study area environment, followed by dogs as the next largest source. This study shows *Giardia* prevalence is similar for humans living in rural and urban settings, but different for animals and that exposure from infected cattle and dogs may be an important public health concern in Coastal Odisha.

1763

COMPARATIVE GENOMIC ANALYSIS OF *COCCIDIOIDES* AND RELATED SPECIES

Christian Olsen, Kashef Qaadri

Biomatters, Newark, NJ, United States

Dimorphic fungi *Coccidioides immitis* and *C. posadasii* are primary pathogens of immunocompetent mammals, including humans. *Coccidioides* infection results from environmental exposure, which is believed to grow as a soil saprophyte in arid desert environs. To investigate hypotheses about the evolution of *Coccidioides*, the genomes of several Onygenales, a close, nonpathogenic relative, and a more diverged pathogenic fungus, were compared with those of 13 more distantly related Ascomycetes. This poster aims to identify shifts in gene family size associated with a host/substrate shift from plants to animals in the Onygenales. Comparison among Onygenales revealed distinct evolutionary changes in *Coccidioides* that may underlie its infectious phenotype, coccidioidomycosis. Phylogenetic analysis suggest that *Coccidioides* species are not soil saprophytes as previously hypothesized. Data indicate that they have evolved to remain associated with their dead animal hosts in soil. Using a bioinformatics workflow, we show that metabolic pathway genes, membrane-related proteins, and putatively antigenic compounds have evolved in response to interaction with an animal host.

1764

MODELING *BRUCELLA* INFECTION DYNAMICS IN PASTORALIST COMMUNITIES: THE ROLE OF HERD MANAGEMENT AND SPECIES COMPOSITION IN SUSTAINING *BRUCELLA* TRANSMISSION

Peter S. Larson, Johannes Foufopoulos, Rebecca Hardin, Annie Wang, **Joseph N. Eisenberg**

University of Michigan, Ann Arbor, MI, United States

Zoonotic bacterial pathogens present significant threats to human health and compromise the economic well-being of pastoralist herding communities. The dynamics of transmission of pathogens such as *Brucella* sp., the causative agent of Brucellosis, however, are poorly understood. Prior studies have suggested that transmission of Brucellosis cannot be sustained in the small to medium size herds characteristic of most pastoralist systems. These analyses, however, did not consider the joint effects of animal trading between herds and inter-species mixing within herds. Using quantitative and ethnographic data collected in a pastoralist community in Laikipia, Kenya, we create a deterministic SIR model of *Brucella* sp. transmission to examine how these transmission heterogeneities may facilitate disease persistence. We explore dynamics of infection given various herd sizes and management strategies observed in Laikipia Kenya. Specifically, we model a community that engages in multispecies livestock raising, assuming different types and levels of interaction between herds. We find that transmission is unsustainable in small herds of all species when in isolation, confirming prior research. Multi-species herds which include a high proportion of goats compared with large stock such as cattle can sustain itself for a longer time, but not indefinitely. Links through sales and purchases or other between-herd contact, however, create conditions where *Brucella* can transmit indefinitely. Though it is likely that herding strategies historically accommodate potentially devastating risks to herd health, it is possible that herders are unaware of threats, both to health and economic wellbeing, presented by bacterial infections such as Brucellosis. In addition to education programs on specific diseases, herders should be encouraged to cull animals showing obvious signs of illness as soon as possible and should be discouraged from selling such animals to minimize risks to human health.

1765

MARKETS AS HUBS OF RISKY CONTACT BETWEEN HUMANS AND WILD/DOMESTIC ANIMALS: CASE STUDIES FROM REPUBLIC OF CONGO (ROC) AND DEMOCRATIC REPUBLIC OF CONGO (DRC)

Jariseta Rambeloson Zo[1], Henri Banga-Mboko[2], Roger Albert Mbété[2], Lonna Shafritz[1], Tula Michaelides[1], Jean-Luc Hornick[3], Susan Zimicki[1]

[1]FHI 360, Washington, DC, United States, [2]Veterinarian and Zoonotic Research Center, Brazzaville, Republic of the Congo, [3]University of Liege, Liege, Belgium

Traditionally, market studies have focused on elements of market dynamics such as the volume of wildlife and domestic animals being sold. We are examining markets through a different lens: to assess them as locations where humans - consumers, vendors, managers - are at increased risk of transmission of zoonotic infections via exposure to live animals, animal products, and poor biosecurity practices. USAID's PREVENT Project is studying eight urban markets in Brazzaville and two in Dolisie, ROC, as well as eight markets in Kinshasa, DRC. We conducted key informant interviews, consumer exit interviews and then a household survey in the market catchment areas (as determined by exit interviews) to learn where people shop, what they buy and why. In addition, since February 2014 we have carried out monthly one-week full-day observations of vendor stalls selling bush meat and/or poultry. This presentation will describe what we have learned to date about hygiene conditions, infrastructure, and biosecurity practices in these markets and about the importance, diversity and seasonal variation of the bush meat trade as well as how the forms of animals sold (live, freshly dead, large pieces, small pieces, smoked, raw) and change with the length of time they are in the market. We will discuss the implications of all of these factors for human exposure and risk

1766

MASSTAG PCR DETECTION OF EV-D68, RSV-A AND B, AND MORE, IN CLUSTERS OF UNEXPLAINED ACUTE FEBRILE ILLNESS IN CAMBODIA

Nary Ly[1], Rafal Tokarz[2], Nischay Mishra[2], Stephen Sameroff[2], Komal Jain[2], Agus Rachmat[1], Sam An Ung[3], Touch Sok[3], Steven Newell[1], Ian Lipkin[2], **Dustin J. Harrison**[1]

[1]U.S. Naval Medical Research Unit – 2, Phnom Penh, Cambodia, [2]Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York, NY, United States, [3]National Institute of Public Health, Cambodian Ministry of Health, Phnom Penh, Cambodia

Fevers of unknown origin constitute a substantial disease burden on patients in Southeast Asia with the majority of the cases remaining undiagnosed. To expand the breadth of possible infectious pathogens, we used MassTag PCR to test for the presence of 20 bacterial and viral respiratory agents from 85 patients with unexplained respiratory illness representing six disease clusters that occurred in Cambodia between 2006 and 2012. We detected potential pathogens in 62 (73%) of 85 total cases, identifying a virus in 37 patients (44%) and a bacterium in 53 (62%) cases. In a cluster from Kandal province from August 2009, we detected a high frequency of enterovirus 68 and human rhinoviruses. Among 22 cases that occurred during October 2009 in Kampong Speu province, we detected human respiratory syncytial virus B. Finally, a cluster of children < five years of age from the Ratanakiri province previously diagnosed with pneumonia, revealed infection from human respiratory syncytial virus A. These findings provide insight into the etiologies of previously undiagnosed acute febrile illness in Cambodia and point to the utility of multiplexed diagnostics during disease outbreaks.

1767

DIAGNOSTIC VALUE OF CLINICAL FEATURES FOR DIAGNOSING PNEUMONIA IN CHILDREN UNDER FIVE YEARS OF AGE: A SYSTEMATIC REVIEW AND META-ANALYSIS

Clotilde Rambaud Althaus[1], Fabrice Althaus[2], Blaise Genton[1], Valérie D'Acremont[1]

[1]Swiss Tropical and Public Health Institute, Basel, Switzerland, [2]Department of Ambulatory Care and Community Medicine, Lausanne, Switzerland

The aim of this review is to assess the diagnostic value of clinical signs and symptoms in identifying radiological pneumonia in children under the age of five and to review the accuracy of WHO criteria for diagnosing clinical pneumonia in developing countries. Electronic databases (Medline and Embase) and reference lists of relevant studies were searched to identify articles assessing clinical predictors of radiological pneumonia in children. 1697 potentially relevant studies were identified. Selection was based on: design (diagnostic accuracy studies), target disease (pneumonia), participants (children below 5 years), setting (ambulatory or hospital care), index test (clinical features), reference standard (chest radiography). Quality assessment was based on the 2011 Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) criteria. For each index test, sensitivity and specificity were calculated. Meta-analyses with the Bivariate model and hierarchical SROC plots were done for index tests assessed in four or more studies. Eighteen articles were included in the analysis. Age-related fast breathing (six studies, pooled sensitivity: 0.62 [95%CI 0.26-0.89]; specificity: 0.59 [0.29-0.84]) and lower chest wall indrawing (four studies, 0.48 [0.16-0.82]; 0.72 [0.47-0.89]) showed poor diagnostic performance in the meta-analysis. Features with the highest pooled, positive likelihood ratios were: respiratory rate above 50/min (1.90 [1.45-2.48]), grunting (1.78 [1.10-2.88]), chest indrawing (1.76 [0.86-3.58]), and nasal flaring (1.75 [1.20-2.56]). Features with the lowest pooled negative likelihood

ratio were: cough (0.30 [0.09-0.96]), history of fever (0.53 [0.41-0.69]), and respiratory rate above 40/min (0.43 [0.23-0.83]). No single clinical feature was sufficient for definitively diagnosing pneumonia. Combining clinical features in a decision tree may improve diagnostic performance, but the addition of new point-of-care tests for diagnosing bacterial pneumonia would help to reach an acceptable level of accuracy.

1768

COMMUNITY ACQUIRED PNEUMONIA IN ADULT HOSPITAL ADMISSIONS NORTHERN VIETNAM; CLINICAL FEATURES AND ETIOLOGY

Behzad Nadjm[1], Ta Thi Dieu Ngan[2], Vu Quoc Dat[2], Rogier van Doorn[1], Peter Horby[1], Nguyen Van Kinh[2], Heiman Wertheim[1]

[1]Oxford University Clinical Research Unit, Hanoi, Vietnam, [2]National Hospital for Tropical Diseases, Hanoi, Vietnam

Community acquired pneumonia (CAP) causes an estimated 1 million deaths in Asia each year including 160 000 amongst those aged 15 - 60 years. Knowledge of local etiology is critical to formulating treatment guidelines and vaccination priorities. Despite this studies of the etiology of CAP in South East Asia have been limited. We conducted a study of the causes of CAP in adult patients admitted to 3 hospitals in Ha Noi, Vietnam. Adults with an infiltrate on chest radiography and one of: cough, dypnoea, fever (≥38.3C) or hypothermia (<36.0C), purulent respiratory secretions, bronchial breathing or rales on auscultation, leucocytosis or leucopenia that had not been residing in hospital or a long term care facility in the 14 days prior to onset were eligible for admission. Consenting patients were enrolled and recieved a standardized clinical evaluation. All patients had routine haematology and biochemistry test results recorded and received blood culture, sputum culture and PCR for the following bacterial pathogens: *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, *M. amphoriforme*, *Chlamydophila pneumoniae*, *C. psittaci*, *Legionella pneumophila* and *L longbeachae*. PCR was also performed for 14 respiratory viruses on nasal/throat swabs and/or sputum. Urine was tested for pneumococcal antigen and L pneumophila serogroup 1. In a selection of cases where acute and convalescent serum was available serology for *C. pneumoniae, M. pneumoniae, Orientia tsutsugamushi*, *Rickettsia typhi* and *R. prowazekii* was also performed. Preliminary results only are available at this time, full results should be available in time for presentation. Preliminary results show a case fatality rate (died in hospital or palliative discharge) of 12/116 (10.3%). The rate of positivity for blood culture was low (5/112, 4.5%). Sputum PCR was positive for *S. pneumoniae* in 80/125 cases (64%), *M. pneumoniae* in17/125 (13.6%), *C. psittaci* in 10/125 (8%), *M. amphoriforme* in 5/125 (4%), *C. pneumoniae* and *L. pneumophila* in 1/125 (0.8%) each and there were no cases of *L. longbeachea*. Clinical findings and outcomes will also be explored.

1769

PROFILE OF *MYCOBACTERIUM TUBERCULOSIS* DRUG RESISTANCE IN A TROPICAL REGION OF PERU 2007-2013

J. Antonio Grandez-Urbina, J. Gonzalo-Rodriguez, S. Bedoya-Vidal, G. Grandez-Castillo, M. Sotelo, A. Santillan-Romero, R. Pichardo-Rodriguez, J. Oscar Tarazona-La Torre, Enver Terrazas Nuñez

Universidad Ricardo Palma, Lima, Peru

Tuberculosis in Peru is a public health problem . In terms of South America is the second country in prevalence after Bolivia, and in America is the third after Haiti and Bolivia . In 2013 , WHO reports that in Peru Multidrug-resistance Tuberculosis (MDR - TB) exceeds the reported cases that year by Colombia , Ecuador , Argentina , Chile and the United States throughout its territory , and ranks first in report for MDR - TB and Extreme drug resistance tuberculosis(XDR - TB) . In Peru the regions with high prevalence of TB are Lima, Callao , Madre de Dios , but the profile of Mycobacterium tuberculosis drug resistance is not known in the region of Madre de Dios . Determine the profile of Mycobacterium tuberculosis drug resistance in patients admitted to retreat in Madre de Dios , Peru , 2007-2013 Observational, cross sectional study . Logbook and monitoring of TB patients in retreatment Regional Health Direction - Madre de Dios of 2007-2013 , included 111 patients diagnosed with tuberculosis smear (+) , they were tested for sensitivity at the National Institute of Health (NIH) . Frequencies established variables, measures of central tendency and dispersion in qualitative variables were used. The frequency of monoresistance 33.63 % (n = 37) , poliresistencia 9.09 % (n = 10) , 45.45 % MDR - TB (n = 50) and 12.72% sensitive cases (n = 14) . The overall frequency of isoniazid resistance was 90.18 % (n = 92) , rifampicin 79.09 % (n = 87) , Streptomycin 18.18 % (n = 20) Etambutol 11.81 % (n = 13) and pyrazinamide 7.27% (n = 8).The initial cultures were basciloscopias negative and 44.6% (n = 50) and 32.1% (n = 36) respectively. Only 1.8% (n = 2) showed HIV positive reaction. . In the present study we found that there is a high prevalence of resistant TB in patients admitted to retreat in the Madre de Dios Region where HIV positive reaction is not related to the presentation. MDR-TB were the most frequent type of resistance and isoniazid is the drug resistance most often generated in monoresistencia patients .

1770

INFLUENZA VACCINE EFFECTIVENESS IN THE TROPICS: MODERATE PROTECTION IN A SURVEILLANCE POPULATION IN BANGKOK BETWEEN AUGUST 2009 AND JANUARY 2013

Jens W. Levy[1], Sriluck Simasathien[2], Veerachai Watanaveeradej[2], Piraya Bhoomiboonchoo[1], Stefan Fernandez[1], Richard G. Jarman[3], Chonticha Klungthong[1], Robert V. Gibbons[1], Piyarat Suntarattiwong[4], **In-Kyu Yoon**[1]

[1]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [2]Phramongkutklao Hospital, Bangkok, Thailand, [3]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [4]Queen Sirikit Institute for Child Health, Bangkok, Thailand

Influenza in the tropics occurs year round with peaks that correspond variably to temperate regions. However, data on influenza vaccine effectiveness (VE) in the tropics is sparse. We report on the effectiveness of influenza vaccine to prevent medically attended laboratory confirmed influenza from a sentinel surveillance study conducted at a Thai military medical facility in Bangkok, Thailand from August 2009 to January 2013. Patients ≥6 months old presenting with influenza-like illness underwent nasal/throat swabs which were tested by influenza RT-PCR. A case test-negative study design was used to evaluate VE. Of 2992 samples available for analysis, 1058 (35.4%) were PCR-positive (cases) and 1934 (64.6%) were PCR-negative (test-negative controls). Five hundred and eight (16.9%) of these patients reported being vaccinated within the previous 12 months. Periods of high and low influenza activity were defined based on publicly available Thai Ministry of Public Health data. Overall adjusted VE was found to be 51.6% (95%CI: 36.8, 63.1%). Adjusted point estimate for VE was highest in the 18-49 year age group (77.0%) followed by 6-23 months (55.1%) and 2-17 years (44.6%). Adjusted estimates were not done for those ≥50 years of age due to small numbers. VE in patients with underlying disease was 75.5% compared to 48.6% in those without. VE appeared to be much higher during high versus low influenza activity periods. Among those who reported receiving vaccine 14 days-3 months prior to illness, VE was 55.8% (95% CI 26.6 to 74.1%), and tended to decrease as the interval between vaccination and illness increased (46.8% at >3 to 6 months; 48.9% at >6 to 9 months; 31.5% at >9 to 12 months). Our findings demonstrate moderate protection by influenza vaccination and support the utility of influenza vaccination in the tropics including in very young children and those with underlying disease. Our study also suggests that booster vaccination may be useful within several months even when the formulation does not change.

1771

SEASONAL INFLUENZA'S ASSOCIATION WITH SPECIFIC HUMIDITY IN THREE TROPICAL CENTRAL AMERICAN COUNTRIES: HONDURAS, NICARAGUA AND COSTA RICA

Radina P. Soebiyanto[1], Wilfrido A. Clara[2], Jorge Jara[3], Maria L. Matute[4], Angel Balmaseda[5], Jenny Lara[6], Mariel Lopez Moya[6], Marc-Alain Widdowson[7], Eduardo Azziz-Baumgartner[7], Richard K. Kiang[1]

[1]NASA Goddard Space Flight Center, Greenbelt, MD, United States, [2]Centers for Disease Control and Prevention, Regional Office for Central America Region, Guatemala City, Guatemala, [3]Universidad Del Valle de Guatemala, Guatemala City, Guatemala, [4]Ministry of Health of Honduras, Tegucigalpa, Honduras, [5]Ministry of Health of Nicaragua, Managua, Nicaragua, [6]Costa Rican Institute for Research and Education on Nutrition and Health, San Jose, Costa Rica, [7]Centers for Disease Control and Prevention, Atlanta, GA, United States

Studies have demonstrated the association between seasonal influenza & meteorological factors. We previously showed that seasonal influenza in Guatemala, El Salvador & Panama was associated with specific humidity. In this work, we investigated the association in 6 departments from three other Central America countries: Cortes & Francisco Morazan in Honduras; Managua in Nicaragua; Alajuela, Cartago & San Jose in Costa Rica. As an indicator for influenza activity, we used the weekly proportion of samples which tested positive for influenza in the period 2008-2013 from each country's National Influenza Centers. Respiratory samples were collected from case-patients presenting with influenza-like illness or severe acute respiratory infection. Meteorological factors - rainfall, temperature & specific humidity (SH) - were obtained from NASA's satellites & models. We used logistic regression and adjusted for previous influenza activity & co-circulating viruses (respiratory syncytial virus, adenovirus and parainfluenza virus). We found that SH was proportionally associated ($p<.05$) with influenza activity in all departments (Odds Ratio (OR)=1.2-1.6). Temperature was inversely associated with influenza activity in Alajuela of Costa Rica (OR and 95% Confidence Interval=0.7(0.6-0.8)) & Cortes of Honduras (OR=0.8(0.7-0.9)). There was no statistical association ($p<.05$) with rainfall in any locations. Among the meteorological factors, SH had the highest contribution (2-15%) to the model in all locations except in Cortes. The model estimated influenza activity accurately (R=0.6-0.9) for the final 6 months in all countries except Honduras. Time-frequency analysis using Hilbert-Huang Transform showed that seasonal components of influenza activity was positively correlated with SH (R=0.2-0.6, $p<.05$), further corroborated the SH findings from logistic regression. Our results highlighted influenza's proportional association with SH in these countries, which was consistent with other studies in the tropics. Understanding of climate role in influenza may help in estimating epidemic timing and intensity.

1772

THE ECONOMIC BURDEN OF VALLEY FEVER IN CALIFORNIA

Leslie Wilson[1], Jie Ting[1], Michael MacLean[2], Michael Peterson[3], Nathan Stockamp[3], Robert Libke[3], Paul Brown[4], Herbert Boro[5], Harold Lin[5]

[1]University of California San Francisco, San Francisco, CA, United States, [2]Kings County Health Department, Kings County, CA, United States, [3]University of California San Francisco Fresno, Fresno, CA, United States, [4]University of California Merced, Merced, CA, United States, [5]Kaiser Permanente-Fresno, Fresno, CA, United States

Coccidioidomycosis or Valley Fever (VF) is a mycotic disease endemic to southwestern US. In California, where 33% of VF cases nationwide occur, VF incidence increased from 4.2-10.8/100,000 in the period 2002-2012 (157% increase). There is no complete study of the economic burden of VF to guide clinical care and public health planning. We estimated the total and per person lifetime direct and indirect cost of VF in 2012 US $. The 4,904 incident VF cases reported in 2012 in California were symptomatic VF infections, which we followed for lifetime costs. Unreported cases were assumed to be asymptomatic infections with no costs. We included early treatment costs of VF misdiagnosed as community acquired pneumonia. We costed VF by disease categories; (uncomplicated pneumonia [UP], chronic/diffuse pneumonia with [CD] and without dissemination [CN], chronic pulmonary nodule [PN], and chronic pulmonary cavity [PC]). Direct costs were VF diagnosis, treatment, and follow-up including physician visits, ER, hospitalization, tests, procedures, and medications. VF epidemiology data were from literature and expert interviews. Treatment and utilization were from published guidelines and 2 hour prepared interviews with 5 expert VF physicians. Hospitalizations were from the 2012 California Patient Discharge Dataset and HCUP prices, medication costs from average wholesale price minus 17% for contract pricing, physician visit were costed using CPT based Medicare estimates. Total lifetime costs of 2012 VF incident cases in CA was $212 million (M), $51,743/person. UP accounted for 85% of our population and 31% of direct lifetime costs ($65.4M), CD 2.5% and 42% costs ($89.2M), CN 2.5% and only 11% of costs, pulmonary nodules and cavities 10% and 16% of costs, respectively, primarily due to cost of differential diagnosis of cancer. Short term work loss costs were $6.4 M, and mortality another $126 M. VF causes a large cost burden, especially for disseminated cases. Variation exists by geographic regions, insurance status and practice patterns. Areas for cost control exist.

1773

THE NEED FOR ENHANCED LABORATORY BASED DIAGNOSIS CAPACITY IN THE TESTING AND SURVEILLANCE FOR OTHER RESPIRATORY VIRUSES FROM PATIENTS REPORTING WITH INFLUENZA LIKE ILLNESSES

Nicholas Owor, Barbara Namagambo, Timothy Byaruhanga, John T. Kayiwa, Richard Chiza, Irene Nabukenya, Barnabus Bakamutumaho, Julius Lutwama

Uganda Virus Research Institute, Entebbe, Uganda

The capacity of Laboratories to promptly identify particular strains or subtypes of organisms using modern diagnostic techniques has become essential for rapid and efficient response to disease outbreaks and preventing potential epidemic or pandemic spread. In Influenza surveillance, patients present with symptoms that match the case definition of Influenza Like Illnesses (ILI) and Severe Acute Respiratory Infection (SARI). Up to 24% of the samples collected and tested for influenza at the National Influenza Centre are positive for influenza while the biggest percentage are negative. There are various respiratory viruses and bacteria that affect humans which include adenovirus, human rhinovirus A, coronavirus OC43, parainfluenza virus 1, parainfluenza virus 3, respiratory syncytial virus B, human metapneumovirus, respiratory syncytial virus A, parainfluenza virus 2 and coronavirus 229E. Nasopharyngeal and oralpharyngeal swab specimens collected from patients presenting with ILI and SARI in 8 sentinel sites between December 2011- April 2014 in Uganda were tested for Influenza by RT- PCR, subtyping and isolation. Of a total of 5931 samples tested; 645(10.9%) were positive for Influenza with 2(0.3%) co-infections, 405(62.8%) Influenza A and 238(36.9%) Influenza B; though, 5286(89.1%) were negative yet the patients presented with symptoms that match the case definition for ILI and SARI. This raises a need to establish baseline information on the prevalence of other respiratory pathogens that cause upper and lower respiratory disease in populations through strengthening laboratory diagnostic capabilities for the identification and characterization of infectious agents likely to cause public health emergencies. There is need for more-simplified testing systems that enable researchers and clinicians to perform multiplexed molecular diagnostics quickly and easily. The results would be useful to guide future surveillance and case management strategies involving other respiratory infections in Uganda.

1774

EPIDEMIOLOGY OF RESPIRATORY VIRAL PATHOGENS FROM SENTINEL SURVEILLANCE IN WESTERN CAMBODIA NEAR THE BORDER WITH THAILAND

Ilin Chuang[1], Ans Timmerman[1], Melanie Melendrez[2], Youry Se[3], Nou Samon[3], Nichapat Uthaimongkol N[1], Stuart Tyner[4], Rith Sareth[5], Rick Jarman[2], Delia Bethell[6], Nitima Chanarat[1], Julie Pavlin[7], Tippa Wongstitwilairoong[1], Piyaporn Saingam[1], But Sam El[3], Sok Touch[8], Ly Sovann[8], Lon Chanthap[9], Stefan Fernandez[1], Phillipe Buchy[5], David Saunders[1]

[1]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [2]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [3]Armed Forces Research Institute of Medical Sciences, Battambang, Cambodia, [4]U.S. Army Institute of Surgical Research, Houston, TX, United States, [5]Institute of Pasteur, Cambodge, Phnom Penh, Cambodia, [6]Department of Paediatrics, Oxford, United Kingdom, [7]Armed Forces Health Surveillance Center, Silver Spring, MD, United States, [8]Communicable Disease Control Department, Phnom Penh, Cambodia, [9]Armed Forces Research Institute of Medical Sciences, Phnom Penh, Cambodia

Little is known about influenza and other common respiratory viruses in remote populations along the Thai-Cambodia border in Western Cambodia. Real-time PCR for influenza was performed on combined nasal and throat specimens from outpatients presenting with influenza-like-illness (ILI) at 4 sentinel sites in Western Cambodia between May 2010 and December 2012; a subset was further characterized by antigenic analysis, antiviral susceptibility testing and full genome sequencing for phylogenetic analysis. PCR-negative ILI-specimens for influenza were cultured; cultured negative specimens were then tested with RT-PCR for enteroviruses and rhinoviruses (EV/RV) and enterovirus EV71. Among 586 ILI-patients (median age 5 year, range 1-77 years), 168 (29%) tested positive for influenza by RT-PCR and at least 1 respiratory virus was detected in 258 (44%) patients. Influenza strains were highly related and matched circulating strains and although vaccination coverage was low, most strains matched the vaccine strains. No intrasubtype reassortment was detected. Our Western Cambodian H1N1(2009) isolates were more closely related (based on full genome analysis) to 10 earlier isolates from Cambodia (94.4% genome conservation) compared to 13 Thai isolates (75.9% genome conservation. Aside from adenovirus (5.74%) and parainfluenza virus (3.8%), detection of non-influenza viruses by viral culture was low (<10%), with no detection of coronavirus, human bocavirus, human metapneumovirus and respiratory syncytial virus. We detected 5.9% of non-polio enteroviruses among our culture-negative specimens: human Coxsackievirus types A4, A6, A8, A9, A12, B3, B4 and human echovirus types E6 and E9. We conclude that influenza epidemiology in this sample of isolates is following similar trends as observed elsewhere in Cambodia. Further research to clarify the burden of adenovirus and non-polio enteroviruses as etiologic agents for acute respiratory infections is needed in Cambodia.

1775

DIFFERENT FROM EQUATORIAL BRAZIL, SOUTHERN HEMISPHERE WHO VACCINATION RECOMMENDATIONS ARE ADEQUATE FOR MOST OF SOUTHERN PARTS OF BRAZIL

Wladimir J. Alonso[1], Priscila S. Born[2], Paola C. Resende[2], Gonzalo Bello[3], Fernando C. Motta[2], Marilda M. Siqueira[2]

[1]Division of International Epidemiology and Population Studies, Bethesda, MD, United States, [2]Respiratory Viruses and Measles Laboratory, Rio de Janeiro, Brazil, [3]AIDS and Molecular Immunology Laboratory, Rio de Janeiro, Brazil

Influenza vaccination is the most important public health measure to prevent severe cases and deaths due to influenza infection. However, influenza viruses are constantly evolving, forcing continuous vaccine reformulation. In this study we investigated the annual genetic matching between circulating influenza viruses and recommended A(H3N2) vaccine components for over a decade (1999-2012) in three regions of a southern hemisphere country with continental dimensions (Brazil). A total of 237 hemagglutinin sequences from Northeast (NE), Southeast (SE) and South (S) Brazilian regions were compared against the corresponding vaccine strains recommended annually by the WHO. We used MEGAv5.1 to infer nucleotide and amino acid distances between sequences and annual vaccine prototypes. PhyML was used for phylogenetic reconstructions by Maximum Likelihood (ML) to infer the antigenic relationship between viral samples and vaccine composition. We next compared the putative effectiveness of the influenza vaccination in the three regions using hypothetical vaccination scenarios where alternative vaccine delivery timing and vaccine compositions (either Southern or Northern Hemisphere WHO recommendations) were considered (comparison following method of Mello et al 2009). We found that, although influenza circulates in most (NE) or all (S,SE) months of year, the current Southern Hemisphere recommendation in Brazil is adequate for these regions. This was less expected in the NE region, but we attribute it to the fact that most of the samples of the NE actually came from the its southernmost part (Bahia, at approximately 13oS), where influenza seasonality differ from the equatorial pattern of circulation. Our results show that WHO hemisphere vaccine recommendation decisions must consider differences in influenza circulation patterns even within regions of a country.

1776

CONCORDANCE BETWEEN SOLID AND LIQUID CULTURE FOR ANTITUBERCULOSIS DRUG SUSCEPTIBILITY TEST (DST) IN PERU

Giancarlo A. Valle[1], Renato Quispe[1], Tatiana Cáceres[1], Eduardo Gotuzzo[2], Carlos Zamudio[1], Carlos Seas[2]

[1]Instituto de Medicina Tropical "Alexander von Humboldt", Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Departamento de Enfermedades Infecciosas, Tropicales y Dermatológicas, Hospital Nacional Cayetano Heredia, Lima, Peru

Löwenstein Jensen (LJ) has been the most widely technique for MTB detection and DST. However, its long incubation and time to detection has led to develop alternative methods. Automated Mycobacteria Growth Indicator Tube (MGIT) constitutes a rapid alternative with comparable results, but manual MGIT (mMGIT) is favorable in low-resources areas because its lower cost. This study evaluates the concordance for DST of LJ and manual MGIT in a country with one of the highest prevalence of tuberculosis in the Americas. Sputum samples collected from respiratory TB suspects, enrolled in diagnostic trials during 2007-2011 in Lima (Capital of Peru), underwent LJ and MGIT. Only samples with positive Capilia test for Mycobacterium tuberculosis (MTB) were included in the DST analysis. Resistance to Isoniazid (INH), Rifampin (RIF), Streptomycin (SM) and Ethambutol (EMB), as well as resistance to INH plus RIF (MDR) were evaluated by Proportion Method (PM) in LJ medium and SIRE system in MGIT. Comparison between performance of MTB detection and susceptibility patterns were assessed by Kappa indices. DST in both solid and liquid mediums was performed in 319 samples. PM-LJ and SIRE-MGIT detected resistance to INH: 21.6% (69/319) and 20.7% (66/319) (Kappa=0.92, p<0.001); RIF: 12.0% (38/319) and 11.0% (35/319) (Kappa = 0.94, p<0.001); SM: 26.3% (84/319) and 25.1 (80/319) (Kappa = 0.82, p<0.001); and EMB: 11.9% (38/319) and 9.4% (30/319) (Kappa = 0.77, p<0.001). Furthermore, PM-LJ and SIRE-MGIT found 10.0% (32/319) and 8.8% (28/319) as MDR cases, respectively (Kappa = 0.89, p<0.001). Manual MGIT emerges as a faster DST alternative to LJ in low resources settings like Peru, with optimal concordance between LJ and mMGIT DST.

1777

COMPARATIVE PERFORMANCE OF WEEKLY TELEPHONE CALLS VERSUS WEEKLY HOME VISITS TO IDENTIFY CASES OF INFLUENZA-LIKE ILLNESS AMONG A COHORT OF PREGNANT WOMEN - GUATEMALA, 2013

Chris Bernart[1], Maria R. Lopez[1], Marvin Xuya[1], Juan C. Moir[2], Saad B. Omer[3], Jorge Jara[1], Eduardo Azziz-Baumgartner[4], Joe P. Bryan[4], John P. McCracken[1]

[1]Universidad del Valle, Guatemala, Guatemala, [2]Guatemalan Ministry of Health, Guatemala, Guatemala, [3]Emory University, Schools of Public Health and Medicine and Emory Vaccine Center, Atlanta, GA, United States, [4]Global Disease Detection Branch, Division of Global Health Protection, Atlanta, GA, United States

Influenza-like illness (ILI) is a leading cause of illness globally. A substantial proportion of ILI is not detected by health services-based surveillance because not all persons with ILI seek healthcare; this may be exacerbated in settings where healthcare access or utilization is limited. Active surveillance through household visits can detect most ILI cases, but is costly and labor-intensive. We followed a cohort of pregnant women in rural Quetzaltenango, Guatemala for ILI (fever and cough or sore throat) by using either weekly phone calls or home visits. Women with < 20 weeks gestation were randomized 1:1 to either a weekly phone call or home visit. Staff attempted up to 3 contacts per week to administer a study questionnaire for ILI symptoms. Probable ILI cases identified by the call or visit were then evaluated within 24 hours by a nurse during a home visit. Participants with ILI had a nasopharyngeal swab collected which was tested by polymerase chain reaction for respiratory syncytial virus (RSV), human metapneumovirus, influenza A/B, parainfluenza virus 1/2/3, and adenovirus. During May-November 2013, 167 women were enrolled, of whom 85 (51%) were randomized to weekly phone calls and 82 (49%) to home visits. Surveillance was completed for 864 (63%) of the 1,364 expected person-weeks of follow-up by phone calls versus 1,010 (73%) of the 1,381 expected person-weeks by home visits (p=0.01). Weekly follow-up identified 9 ILI cases in the phone call group versus 13 ILI cases in the home visit group (p=0.5 for ILI). We detected 3 infections in the phone call group (adenovirus, parainfluenza-2, RSV) versus 6 infections in the home visit group (RSV, flu-B, parainfluenza types 2 and 3) (p=0.8). Although more costly and time consuming, home visits were more likely to have successfully completed questionnaires than phone calls. The sample size was inadequate to determine the difference in detection of lab-confirmed ILI cases between visits and calls. The choice between phone calls and home visits to identify case-patients will depend on the surveillance objectives and available resources.

1778

ATTEMPTED ALTERNATIVE METHODS FOR THE DIAGNOSIS OF *PNEUMOCYSTIS JIROVECI* PNEUMONIA (PCP) IN HIV/AIDS PATIENTS IN RESOURCE-LIMITED SETTINGS

Melissa Reimer-McAtee[1], Meredith Lora[2], Carolina Mejia[3], Monica Pajuelo[4], Faustino Torrico[3], Daniel Lozano Beltran[3], Robert Gilman[5]

[1]Tulane University, New Orleans, LA, United States, [2]University of San Francisco California, San Francisco, CA, United States, [3]Universidad San Simon, Cochabamba, Plurinational State of Bolivia, [4]Universidad Peruana Cayetano Heredia, Lima, Peru, [5]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Despite the increased use of prophylactic therapy and improved access to antiretroviral therapy, *Pneumocystis jiroveci* pneumonia (PCP) remains one of the most common life-threatening opportunistic infections in HIV-infected patients worldwide. Difficulty obtaining an adequate sputum sample is often a barrier to the diagnosis of PCP in sputum-scarce patients where broncheoalveolar lavage is not available. This study examined the rates of PCP in HIV patients with respiratory symptoms in Cochabamba, Bolivia, and evaluated detection of PCP in samples other than sputum in an attempt to overcome the challenge of sample collection for the diagnosis of PCP in resource-limited settings. Fifty-one HIV patients admitted to the hospital for respiratory symptoms in Cochabamba, Bolivia in 2010 were enrolled in the study. In addition to induced sputum, an oral rinse, stool, and gastric secretion sample obtained by the string test were collected from each subject. Presence of PCP was evaluated by real-time quantitative PCR in a laboratory in Lima, Peru after the completion of sample collection. Of the 51 induced sputum samples collected, seven (13.7%) were positive for PCP. The oral rinse and string test each detected PCP in one of the samples. PCP was not detected in any of the stool samples. The mortality rate of those diagnosed with PCP was 42.9% (3/7). Although other studies have reported the ability to diagnose PCP by the use of PCR on oral rinse samples, our study was not able to repeat this. Additionally, we were not able to detect PCP in either stool or gastric secretion samples obtained by the string test (as has been used for the detection of Mycobacterium tuberculosis in other studies). Our study does show that PCP is a cause of substantial morbidity in the HIV population in Bolivia, and it is possible that there were additional cases of PCP that went undiagnosed in our study because of we were limited to induced sputum. Thus, improved diagnostic modalities are needed for the detection of PCP in resource-limited settings (delete all in italics). Although the rates of PCP in our study, and in most resource-scarce settings, are significantly less than tuberculosis, our study shows that PCP is still a cause of substantial morbidity and mortality in the HIV population in Bolivia. We were not able to detect PCP in stool samples, and our yield was very low in oral rinse and gastric secretion samples obtained by the string test. It is possible that there were additional cases of PCP undiagnosed in our study because we were limited to samples of induced sputum. Our study highlights the fact that improved diagnostic modalities are needed for the detection of PCP in resource-limited settings.

1779

HAMSTER WEIGHT PATTERNS PREDICT THE INTENSITY AND COURSE OF *SCHISTOSOMA HAEMATOBIUM* INFECTION

Thien-Linh Le

Stanford School of Medicine, Stanford, CA, United States

Although Syrian golden hamsters are a widely used host for experimental infection by *Schistosoma haematobium*, surprisingly little is known about the associated intensity and course of infection, making the use of these animals potentially unreliable. As such, we sought to define inexpensive, simple, noninvasive, and accurate methods for assessing and predicting the severity of disease in *S. haematobium* infected hamsters in order to prevent premature hamster sacrifice and unexpected morbidity and mortality. Through monitoring the weight and behavior of infected hamsters, we determined that the weight loss patterns of infected hamsters are highly correlated with commonly used measures of the severity of infection (i.e. numbers of eggs passed in the stool and worm burdens). In contrast, we found no significant correlation between hamster weight loss patterns and egg yields from liver and intestinal tissues. Our findings suggest that a more complex relationship exists among worm burden, fecundity, and egg passage in the feces than previously appreciated. Regardless, our data may be useful for workers seeking to optimize harvests of *S. haematobium* eggs and worm pairs from infected hamsters for downstream applications.

1780

LONG DELAY IN DIAGNOSIS AND HIGH LITHIASIS PROPORTION IN SURGERY SUGGEST UNDERESTIMATION OF HUMAN FASCIOLIASIS IN ARGENTINA

Veronica H. Agramunt[1], Roberto l. Mera Y. Sierra[2], Laura Sidoti[2], Maria Dolores Bargues[1], Santiago Mas-Coma[1]

[1]Departamento de Parasitologia, Facultad de Farmacia, Universitat de Valencia, Burjassot-Valencia, Spain, [2]Catedra de Parasitologia y Enfermedades Parasitarias, Facultad de Ciencias Veteriarias y Ambientales, Universidad J.A. Maza, S. Jose, Guaymallen, Mendoza, Argentina

A retrospective overview of human fascioliasis in Argentina highlighted the long delay with which many patients were diagnosed. Calculated delay average of the time elapsed between the appearance of symptoms and confirmation of infection by appropriate diagnosis is very high, of 1262 days, nearly 3.5 years, and there are references about patients having suffered from symptoms for ten or more years without diagnosis. This suggests either infected subjects not looking for professional diagnosis due to mild symptoms of low fluke burdens and/or misdiagnosis of patients due to the non-pathognomonic clinical picture, easily confused with other diseases when the patient attends a health centre not used to dealing with fascioliasis. Moreover, the number of cases in which a surgical procedure contributed to the diagnosis when *Fasciola hepatica* specimens were unexpectedly found upon liver exploration appeared to be surprisingly high. In the majority, surgery was indicated due to abdominal pain and biliary obstruction suggestive of lithiasis. The importance of intraoperative cholangiography was highlighted in cases in which, even though gallstones were removed, evidence of obstruction observed during the cholangiography led to the finding of flukes. In most of these surgical cases with lithiasis suspicion, the patient inhabited a large city (Buenos Aires, Córdoba, Mendoza, Tucuman) as opposed to a rural area where attending a health care centre is less usual due to economic reasons or at least complicated due to the long journey thas has to be made. This additionally suggests a far greater underestimation of the problem in rural areas. There were patients in whom fluke infection was detected only after a second surgical intervention. Both long delay in diagnosis and high lithiasis proportion suggest that many patients are frequently overlooked and pose a question mark about fascioliasis detection in the country.

1781

RNAI OPTIMIZATION IN *FASCIOLA HEPATICA* NEWLY EXCYSTED JUVENILES: LONG DSRNA INDUCE MORE PERSISTENT SILENCING THAN SIRNA

Nicolas DellOca[1], Tatiana Basika[1], Gabriel Rinaldi[2], Ileana Corvo[1], Estela Castillo[3], Paul J. Brindley[2], Jose F. Tort[1]

[1]Departamento de Genética, Facultad de Medina, Universidad de la Republica, Montevideo, Uruguay, [2]Department of Microbiology, Immunology and Tropical Medicine, and Research Center for Neglected Diseases of Poverty, School of Medicine and Health Sciences, The George Washington University, Washington, DC, United States, [3]Sección Bioquímica, Facultad de Ciencias, Universidad de la Republica, Montevideo, Uruguay

The exponential growth of the genomic and transcriptomics knowledge of parasitic flatworms allows the identification of several novel putative genes of unknown function. In trematodes RNA interference emerges as almost the only available tool to analyze gene function since classical genetics or other reverse genetics approaches still remain unavailable. Whereas this approach has been tested in several parasites of this group it has been optimized only in schistosomes likely reflecting the difficulties in the establishment of the technology as a routine tool. In this report we present progress in the optimization of this technology in the liver fluke *Fasciola hepatica*, causative agent of fasciolosis. This disease is one of the most problematic infections affecting livestock worldwide, and the increasing appearance of human cases had lead the WHO to recognized this disease as a reemeging zoonosis. Using a single copy gene encoding leucine aminopeptidase (LAP) as the target, we refined delivery conditions, identifying electro-soaking (electroporation and subsequent incubation) as the most efficient method to introduce small RNAs into the fluke. We observed consistent knock down of LAP with low (2 µg/ml) dsRNA concentrations. While this low concentration may reduce or obviate off-target effects, it also compromise the tracking of the RNAi incorporation by fluorescent labeling. We also tested the effects of long and short interfering RNAs. While both long dsRNA and short interfering RNA (siRNA) are equally effective at inducing a short-term knock down, dsRNA induced more persistent silencing up to 21 days after treatment, suggesting that mechanisms of amplification of the interfering signal can be present in this parasite. Persistent silencing from invasive stage for up to 3 weeks (close to what it takes for the parasite to reach the liver) opens the possibility of using RNAi for the validation of new putative therapeutic targets

1782

PREVALENCE OF *HAPLORCHIS TAICHUI* AMONG HUMANS AND FISH IN LUANG PRABANG PROVINCE, LAO PDR

Jong-Yil Chai[1], Woon-Mok Sohn[2], Tai-Soon Yong[3], Keeseon S. Eom[4], Duk-Young Min[5], Dongmin Lee[4], Bong-Kwang Jung[1], Virasack Banouvong[6], Bounnaloth Insisiengmay[7], Bounlay Phommasack[7], Han-Jong Rim[8]

[1]Seoul National University College of Medicine, Seoul, Republic of Korea, [2]Gyeongsang National University School of Medicine, Jinju, Republic of Korea, [3]Yonsei University College of Medicine, Seoul, Republic of Korea, [4]Chungbuk National University School of Medicine, Cheongju, Republic of Korea, [5]Eulji University School of Medicine, Daejeon, Republic of Korea, [6]Malaria Center, Luang Prabang, Lao People's Democratic Republic, [7]Ministry of Public Health, Vientiane, Lao People's Democratic Republic, [8]Korea University College of Medicine, Seoul, Republic of Korea

This study confirmed the prevalence of the intestinal fluke *Haplorchis taichui* (Trematoda: Heterophyidae) among people and fish in Luang Prabang Province, Lao PDR. Fecal specimens were collected from 559 riparian people (229 males and 330 females), residing in 4 Districts (Luang Prabang, Xieng Ngeun, Pak Ou, and Nam Bak) and were examined by the Kato-Katz fecal smear technique. The overall helminth egg positive rate was 64.9%. The positive rate for small trematode eggs (STE), which may include *H. taichui* and other heterophyids, Opisthorchis viverrini, and lecithodendriids, was 15.2%. For recovery of adult helmniths, 10 STE-positive people were treated with 40 mg/kg praziquantel and 15 mg/kg pyrantel pamoate, and then purged. Mixed infections with 3 *Haplorchis* species (*H. taichui*, *H. pumilio*, and H. *yokogawai*), a species of cestode (*Taenia saginata*), and several species of nematodes including *Enterobius vermicularis* and hookworms were found. The worm load for trematodes was exclusively high for *H. taichui* with an average of 7691 specimens per infected person, followed by *H. yokogawai* (8.3 specimens) and *H. pumilio* (4.1 specimens). Out of 207 freshwater fish (17 species) purchased in a market in Luang Prabang District, 138 (67%) harboured *H. taichui* metacercariae (metacercarial burden per fish; 520). Lower prevalence of fish and lower metacercarial density were observed for *H. yokogawai* (52% and 50 per fish, respectively) and *H. pumilio* (18% and 3 per fish, respectively). STE found in the surveyed population of Luang Prabang Province were verified to be those of intestinal fukes, particularly *H. taichui*.

1783

MOLECULAR MODELING OF ADENYLATE KINASE 1 AND 8 KDA CALCIUM-BINDING PROTEIN OF *CLONORCHIS SINENSIS*

Tai-Soon Yong[1], Tae Yun Kim[1], Eun Joo Chung[2]

[1]Yonsei University College of Medicine, Seoul, Republic of Korea, [2]Korea National Institute of Health, Seoul, Republic of Korea

Allosteric proteins involved in signal transduction transforms their molecular structure by binding co-factors, and thus they have a potential

for new drug development. In this study, two allosteric proteins, adenylate kinase 1 (ADK1) and 8 kDa calcium-binding protein (CaBP) of the Chinese liver fluke *Clonorchis sinensis* were cloned and modeled. *C. sinensis* EST clone Cs63 and Cs296 were cloned and sequenced. To compare them with other proteins of parasites, multiple sequence alignment and phylogenetic analysis were performed. For molecular modeling, both sequences were subjected to SWISS-MODEL. Recombinant proteins generated bacterially were used for their functional analysis. By BLAST search, Cs63 and Cs296 were confirmed as ADK1 and 8kDa calmodulin-like CaBP, respectively, and thus they were named as CsADK1 and CsCa8, respectively. Sequence and hydrophobicity of them were similar to those identified from parasitic helminthes. Molecular model of CsADK1 contained CORE, LID and NMP domains and expected to transform its structure by binding co-factor-like AP5. CsCa8 was predicted to have two distinctive EF-handed calcium-binding sites by molecular modeling. Calcium ion could bind to each of EF-hands of CsCa8 model. Both recombinant proteins were functionally active in biochemical assay. Results obtained from the study provide structural basis of *C. sinensis* ADKs and CaBPs for the development of new anthelminthic drugs.

1784

AN OPEN-LABEL, RANDOMIZED, MULTICENTRIC STUDY OF TRICLABENDAZOLE FOR FASCIOLIASIS IN CHILDREN FROM PERU

Vicente Maco[1], Luis Marcos[1], Jaime Delgado[2], Julio Herrera[1], José Nestares[1], Angelica Terashima[1], Frine Samalvides[1], Eduardo Gotuzzo[1]

[1]Institute of Tropical Medicine Alexander von Humboldt, Lima, Peru, [2]Health Care Center of Progreso, Asillo, Azangaro, Puno, Peru

Human fascioliasis is an important public health problem in Latin America mainly affecting school-aged children in poor areas of the Andean Region (Peru and Bolivia). The optimal therapeutic scheme of triclabendazole (TCBZ), the recommended anthelmintic against the trematode *Fasciola hepatica*, has not been well defined (with a 10 mg/kg single dose being the most common regimen recommended), and clinical trials that assess effectiveness and tolerability in children are scarce. We aimed to evaluate the efficacy and tolerability of 2 therapeutic schemes of TCBZ in different areas of Peru. A total of 84 individuals (mean age ± SD: 9.27± 2.48 years) with *F. hepatica* eggs in their stools (chronic infection) were enrolled in an open-label, phase II clinical trial from areas located along the Peruvian Andes. Individuals were randomly allocated into 2 groups: 44 received 2 dosages of TCBZ at 7.5 mg/kg each, with a 12 h interval post-prandially (group I-tested group), and 40 received a single dose of 10 mg/kg, post-prandially (group II-standard group). The efficacy (parasitological cure) was evaluated by the presence of eggs in stools at regular intervals and up to 60 days post-treatment. Tolerability was evaluated by the presence of clinical symptoms during the first week after TCBZ administration.A parasitological cure was obtained in 100% of individuals from the tested-group, and 95.0% in the standard-group (p>0.05). The most common adverse event was biliary colic, documented in 25.0% in group II (95% CI= [Ed.1] 11.9-38.9) on day 2, and in 20.5% in group I (95% CI= 7.8-33.7) on day 4, possibly related to the expelling of the adult worms through the biliary tract.In conclusion, the tested scheme was highly efficacious (100% cure rate) and tolerable, and it may be an optimal therapeutic scheme for the treatment of fascioliasis in children in Peru. This represents the largest series of children treated with TCBZ in a non-hospital setting and the largest in Peru.

1785

IDENTIFICATION, CHARACTERIZATION AND EVALUATION OF RECOMBINANT ANTIGENS FOR THE DIAGNOSIS HUMAN PARAGONIMIASIS

Peter U. Fischer, Kurt C. Curtis, Kerstin Fischer, Samantha N. McNulty, Makedonka Mitreva, R. Reid Townsend, Gary J. Weil

Washington University School of Medicine, St. Louis, MO, United States

Paragonimiasis is a foodborne trematode infection that affects 23 million people mainly in Asia. The parasite causes chronic cough with fever and hemoptysis, and lung fluke infection is often confused with tuberculosis. We used a systems biology approach to identify antigens that might lead to improved diagnostic tests for this infection. Antibodies from patients with *Paragonimus kellicotti* were used to isolate antigens for proteomic analysis, and RNAseq data from adult worms were used for protein identification. Among the 22 most abundant identified proteins were a number of orthologues to known diagnostic antigen as well as novel candidates. Sequences for these proteins have 80-90% identity with amino acid sequences for orthologues in P. westermani. We expressed five *P. kellicotti* proteins as his-fusion proteins in E. coli, and these were used to raise antibodies in mice. Immunohistology performed with sections of adult worms showed that four of them were localized to the tegument, at the parasite host interface. In contrast, a known egg antigen was absent from the tegument but present in developing and mature eggs. We evaluated the diagnostic potential of these antigens by Western blot with sera from patients with paragonimiasis (from Missouri and the Philippines), fascioliasis, and schisosomiasis and with sera from healthy North American controls. Two recombinant proteins showed high sensitivity and specificity as diagnostic antigens. Antibodies to the egg protein seemed to be specific marker for patients with mature adult worm infections (with Paragonimus ova in stool or sputum). In conclusion, this study has identified and characterized promising antigens for diagnosis of human paragonimiasis.

1786

IMPACT OF *SCHISTOSOMA MANSONI* IN SCHOOL-AGED CHILDREN LIVING IN KASANSA, DEMOCRATIC REPUBLIC OF THE CONGO

Mbuyi M. Kabongo[1], Sylvie Linsuke[2], Sylvain Baloji[3], Wilfried Mukunda[4], Raquel Inocêncio da Luz[3], Christine Stauber[1], Richard Rothenberg[1], Jean Pierre Van geertruyden[3], Katja Polman[5], Marleen Boelart[3], Pascal Lutumba[2]

[1]Georgia State University, Atlanta, GA, United States, [2]University of Kinshasa, Kinshasa, Democratic Republic of the Congo, [3]University of Antwerp, Antwerp, Belgium, [4]Programme National de Lutte contre la Bilharziose et Parasitoses Intestinales, Kinshasa, Democratic Republic of the Congo, [5]Institute of Tropical Medicine, Antwerp, Belgium

Schistosomiasis (SCH) is an important public health problem in developing countries and school aged children are the most affected. The aim of this study was to evaluate the impact of SCH on the population of school-aged children living in the high endemic area of Kasansa HZ in terms of malnutrition, anemia and low school performance. The overall health status of the children was poor with very high prevalence of *S. mansoni* infection (89.3%), malaria infection (65.1%), anemia (61.4%) and stunting (61.0%). School performance was also negatively affected with 54.6% of the children having failed at least one class. Regular contact with river water was the most significant risk factor related to SCH infection. Anemia was influenced by SCH infection (p=0.003) and weak egg load was associated with stunting (p=0.04). However, due to poverty the causality between chronic malnutrition and anemia can be in either direction, potentially aggravated by SCH. Low school performance was mainly influenced by low income (<1 USD). Poverty exacerbated both health and school performance. Control measures are urgently needed to improve the health status of these children with in depth studies to demonstrate the causalities for each of the diseases in this population.

1787

FIELD EVALUATION OF MINI-FLOTAC®, SPONTANEOUS SEDIMENTATION TECHNIQUE IN TUBE OF TELLO, RAPID SEDIMENTATION TECHNIQUE BY LUMBRERAS AND KATO-KATZ FOR THE DIAGNOSIS OF ENTERIC PARASITES IN THE HIGHLANDS OF PUNO, PERU

Vicente Maco[1], Catherine de Beaumont[2], Davide Ianniello[3], Giuseppe Cringoli[3], Quentin Bickle[4], Angelica Terashima[1], Eduardo Gotuzzo[1], Luis Marcos[1]

[1]Institute of Tropical Medicine Alexander von Humboldt, Lima, Peru, [2]Division of Infectious Diseases, Albert Einstein College of Medicine, New York, NY, United States, [3]Department of Pathology and Animal Health, University of Naples "Federico II", Naples, Italy, [4]London School of Hygiene & Tropical Medicine, London, United Kingdom

Enteric parasites show a peculiar distribution along the geography of Peru. Although a low-cost, reliable, local coproparasitological technique such as the Spontaneous Sedimentation Technique in Tube of Tello (SSTT) has been used over decades this has not been compared to standardized methods. Here, we compare the performance of SSTT with the mini-FLOTAC®, a coproparasitological technique under validation, in an area where intestinal multiparasitism and fascioliasis are common. Rapid Sedimentation Technique (RST) by Lumbreras, and Kato-Katz (KK) were included. We conducted a stool survey among school-aged children in Naupa Pampa, Calapampa, and Progreso, located in Azángaro (3859 m) (Puno, Peru). Four techniques, mini-FLOTAC®, SSTT, RST, and KK were performed. The sensitivity and negative predictive value (NPV) of each technique were compared using the combined results of all positive techniques as the "gold standard". The inter-technique agreement (κ) was also evaluated. A p value 10%): *Entamoeba coli* (77.2%), *Blastocystis hominis* (66.5%), *Endolimax nana* (50.3%), *Iodamoeba buetschlii* (15.0%), *Entamoeba histolytica/dispar* (12.0%), *Giardia lamblia* (11.4%), *Fasciola hepatica* (10.8%), and *Chilomastix mesnili* (10.2%). The area of study is hyperendemic for human fascioliasis with high prevalence in Ñaupa Pampa (25%). Mini-FLOTAC® showed a higher performance than SSTT and RST for *H. nana* (13 vs. 11 vs. 8) and *F. hepatica* (15 vs. 10 vs. 8), and higher sensitivities than SSTT, RST and KK for both helminths ($p<0.05$). The use of multiple techniques is an appropriate approach for highly endemic areas where intestinal multiparasitism is common. SSTT is highly sensitive for enteric protozoa, including when compared to standardized techniques such as the mini-FLOTAC®. Mini-FLOTAC® holds promise for the diagnoses of H. nana and *F. hepatica* in endemic areas. Notably, *F. hepatica* infection continues to be highly prevalent among children in Azangaro, thus prompt and realistic field interventions are needed.

1788

GRANULOMATOUS INFLAMMATION OF THE BLADDER COMPROMISES THE OVERLYING UROTHELIAL BARRIER

Deborah Boyett

Stanford University, Stanford, CA, United States

Bladder granulomas can form as a result of retained suture material from surgery, BCG treatment, urinary tuberculosis, and urogenital schistosomiasis. We previously demonstrated in a mouse model of urogenital schistosomiasis that multiple urothelial barrier function genes (i.e., uroplakins) were downregulated on a whole bladder level after bladder wall injection with *Schistosoma haematobium* eggs. Given that egg-injected bladders exhibit hyperplasia of the urothelium overlying resulting egg granulomata, we hypothesized that this hyperplastic response was a response to egg inflammation-induced, regional compromise of the molecular urothelial barrier. Anesthetized mice underwent laparotomies and bladder exposure. Mice then underwent either bladder wall injection with *S. haematobium* eggs or a vehicle control. Five days later, mice were sacrificed and their bladders harvested and fixed. Frozen sections of each bladder were stained with Cresyl violet. Laser microdissection was used to harvest RNA from three regions of each bladder: 1) the "proximal" urothelium (urothelium overlying the egg granuloma site); 2) the "distal" urothelium (urothelium from the opposite side of the bladder relative to the granuloma site); and 3) granuloma tissue (subepithelial). Equal areas were harvested for each of the three tissue sites (478,000-1,000,000 µm2/site). RNA was isolated, reverse transcribed to cDNA, pre-amplified using the NuGen PicoSL WTA System, and then subjected to qPCR for uroplakin and housekeeping genes. Control vehicle-injected bladders exhibited subepithelial edema, normal urothelium, and some inflammation. Egg-injected bladders, in contradistinction, featured markedly thickened, hyperplastic urothelium overlying areas of significant egg-associated inflammation. The proximal urothelium in egg-injected bladders featured lower expression levels of uroplakin genes relative to the distal urothelium. Bladder granulomas, such as those induced by urogenital schistosomiasis, may locally suppress the overlying urothelial barrier. This suppression may be a mechanism by which chronic bladder inflammation results in urothelial-related bladder dysfunction. Future work will further define these mechanisms and their physiologic significance.

1789

INFLUENCE OF HISTONE MODIFYING ENZYMES AND MITOGEN-ACTIVATED PROTEIN KINASE SIGNALING PATHWAY ON *SCHISTOSOMA MANSONI* SURVIVAL AND REPRODUCTIVE DEVELOPMENT

Marina M. Mourão[1], Luiza F. Andrade[1], Livia G. Avelar[1], Juliana A. Geraldo[1], Fernanda S. Coelho[1], Laila A. Nahum[2], Raymond J. Pierce[3], Guilherme Oliveira[1]

[1]Genomics and Computational Biology Group, Instituto Nacional de Ciência e Tecnologia em Doenças Tropicais, Centro de Pesquisas René Rachou, Fundação Oswaldo Cruz – FIOCRUZ, Brazil, Center for Excellence in Bioinformatics, Belo Horizonte, Brazil, [2]Fundação Oswaldo Cruz, Faculdade Infórium de Tecnologia, Belo Horizonte, Brazil, [3]CIIL, Inserm U1019, CNRS UMR8204, University Lille Nord de France, Institut Pasteur de Lille, Lille, France

Praziquantel is an efficacious drug against schistosomiasis, however, there is the risk of drug resistance development and therefore new drugs are necessary. Although, the roles of Histone Modifying Enzymes (HMEs) and Mitogen-activated protein kinases (MAPKs) are unclear in schistosomes, they are increasingly approved as targets for drug development with a rising number of inhibitors under development. In other organisms, HMEs and MAPKs influence a number of tissue-specific biological activities such as cell survival, differentiation and proliferation. Here, we employed RNA interference (RNAi) to elucidate the functional roles of 16 HMEs and 6 genes involved in MAPK signaling pathway in *S. mansoni*. First, the HMEs and ePKs were identified in the predicted proteomes of *Schistosoma mansoni*, *S. japonicum*, and *S. haematobium* by HMM searches. Genes were annotated and selected regarding their putative function in the parasite. One histone deacetylases (HDAC8), 10 methyltranferases (HMTs), 5 demethylases (HDM), SmRas, SmERK1, SmERK2, SmJNK, SmCaMK2, and Smp38 were chosen for experimental validation. RNAi and pharmacological inhibition were used to elucidate the functional role of HMEs and MAPK signaling pathway proteins in *S. mansoni*. Mice were injected with schistosomula subsequent to RNAi and the development of adult worms observed. The data demonstrate that SmHDAC8 and SmJNK contributes to the parasite transformation and survival, whereas HDAC8, PRMT3, KDM1/KDM2, SmERK, and Smp38 seems to be involved in egg production as infected mice had significantly lower egg burdens and female worms presented underdeveloped ovaries. Additionally, SmJNK and Smp38 dsRNA treated worms exhibited tegumental damage. We also observed that Smp38 is involved in the activation of detoxification enzymes. Our results help characterize the importance of HMEs and MAPK pathway in the normal development and survival of the schistosome parasite and suggest some of these enzymes as useful drug targets to prevent schistosomiasis progression.

1790

PROTEOMIC ANALYSIS OF *BIOMPHALARIA GLABRATA* HEMOCYTES DURING ENCAPSULATION OF *SCHISTOSOMA MANSONI* SPOROCYSTS *IN VITRO*

Marília Gabriela dos Santos G. Cavalcanti[1], Grzegorz Sabat[2], Xiao-Jun Wu[2], Utibe Bickham[2], Timothy P. Yoshino[2]

[1]Federal University of Paraíba, João Pessoa, Brazil, [2]University of Wisconsin, Madison, WI, United States

In order to better understand the effector mechanisms associated with parasitic encapsulation reactions, whole hemolymph containing hemocytes was extracted from susceptible (NMRI) and resistant (BS-90) *Biomphalaria glabrata* strains and incubated in the presence or absence of newly-transformed *Schistosoma mansoni* sporocysts (Sp). After 18 h of incubation at 26 C, hemocyte capsules were isolated, frozen *en bloc*, cryo-sectioned and subjected to laser capture microdissection to yield samples enriched for cells involved in hemocyte/Sp reactions for comparison to hemocyte capsules without Sp. Isolated cryosections were analyzed by nanoLC-ESI-MS/MS for peptide isolation and sequencing. Putative protein identifications were made by BLAST analyses vs. the non-redundant NCBI protein database (db) and a 6-frame translated *B. glabrata* protein db in VectorBase. Preliminary analyses revealed at total of 358 putatively identified proteins of which 71 were from *Schistosoma* spp. (mainly *S. mansoni*) and 287 were non-*Schistosoma* sequences. Significantly more larval sequences were identified in NMRI/Sp capsules compared to BS-90/Sp samples, consistent with greater killing and larval rejection typically seen in R hemocyte reactions *in vitro*. After normalizing the dataset across samples using total unique peptide counts for actin and tubulin, other notable immune-related observations were made: (a) A greater reduction of HSP70 peptides in BS-90/Sp capsules compared to NMRI/Sp (67% BS-90 vs. 37% NMRI); (b) Frep2 was the only Frep identified in all samples, and only in the BS-90/Sp sample, implying an upregulation of Frep2 during parasite encounters; (c) An upregulation of extracellular matrix/adhesion proteins (dermatopontin2, HMG1, matrilin2 and α-integrin) only in BS-90/Sp or BS-90 control samples suggesting possible roles in immune-related cell-parasite or cell-cell adhesion reactions; and (d) Enrichment of MnSOD, a potential effector molecule, in the BS-90 hemocyte capsules compared to those of NMRI snails. It is anticipated that continued mining of this rich dataset will yield valuable insights into hemocyte immune function.

1791

COMPARATIVE ANALYSIS OF GENE EXPRESSION IN *SCHISTOSOMA MANSONI*-EXPOSED *BIOMPHALARIA GLABRATA* (BS90 STOCK) SNAILS MAINTAINED AT PERMISSIVE AND NON-PERMISSIVE TEMPERATURES

Matty Knight[1], Ramzi Temanni[2], Wannaporn Ittiprasert[3], Andre Miller[3], Yuan Li[2], Najib M. El-Sayed[2]

[1]George Washington University, Washington, DC, United States, [2]University of Maryland, College Park, MD, United States, [3]Biomedical Research Institute, Rockville, MD, United States

We have shown previously that the refractory phenotype in the *Biomphalaria glabrata* BS-90 stock is a temperature-dependent trait. Thus, using mild non-lethal heat (32°C) to induce stress genes such as Hsp70 and Hsp90 prior to *S. mansoni* infection rendered these normally resistant snails susceptible. In order to determine differences between transcription profiles of these snails responding to early parasite infection when they are ether resistant or susceptible, RNA samples from *Schistosoma mansoni* exposed juvenile BS-90 snails, maintained through several generations either at the permissive (32°C), or non -permissive temperature (25°C) were sequenced. Bioinformatic analyses of RNAseq datasets revealed a preponderance of stress related transcripts in parasite-exposed BS-90 snails maintained at the permissive temperature compared to similarly exposed reference stock maintained at room temperature. For example, at 2 hours post - exposure, a 77- fold induction of Hsp70 transcript was observed in susceptible BS-90 snails maintained at 32°C, corroborating earlier results that showed that this transcript was induced differentially between juvenile resistant and susceptible parasite-exposed snails. Differential expression of other stress genes, Hsp 90 (12-fold induction), Hsp 83 (40-fold induction) and Hsp 68 (5-fold induction) was also detected in these BS-90 snails responding to *S. mansoni* at the permissive temperature. These data, taken together with previous results provides further evidence for the role of stress in the snail- host and schistosome interaction.

1792

IMMUNOMODULATORY PROTEINS OF *SCHISTOSOMA HAEMATOBIUM*

Luke F. Pennington[1], Debalina Ray[2], Chi-Ling Fu[1], Yi-Ju Hsieh[1], Michael H. Hsieh[1]

[1]Stanford University, Palo Alto, CA, United States, [2]University of California San Francisco, San Francisco, CA, United States

Approximately 120 million individuals are infected with *Schistosoma haematobium* in sub-Saharan African alone. *S. haematobium* adult worms lay eggs throughout the urogenital tract that induce a pronounced inflammatory response. To date very little is known about how *S. haematobium* is able to produce such a robust immune response while evading immune clearance and immunity in many individuals. One plausible explanation lies in parasite secreted immunomodulatory proteins, however, due to the historical lack of a robust *S. haematobium* animal model, very little is known about the role of these proteins in urogenital pathology. We have recently cloned a homolog of the IL-4-inducing principle of *S. mansoni* eggs (IPSE), a protein originally identified in *S. mansoni,* the sister species of *S. haematobium* responsible for hepatic and enteric schistosomiasis. *S. mansoni* IPSE has been shown to bind IgE on the surface of basophils in an antigen independent manner, and drive basophil degranulation. To date it is not clear why it is advantageous for the parasite to secrete a protein capable of activating effector cells associated with anti-parasite responses. Intriguingly the *S. haematobium* IPSE homolog shares only 63% identity with its *S. mansoni* counterpart, suggesting that the protein may have evolved to suit each species' infectious niche. Furthermore studies with recombinant *S. haematobium* IPSE and *S. mansoni* IPSE suggest that the immunoglobulin isotype binding profiles of IPSE differ across species. Despite IPSE's sequence divergence and differences in immunoglobulin binding, several important protein features appear to be conserved across species, including a nuclear localization sequence. Using a novel model of *S. haematobium* egg-induced pathology we have shown that immunization with IPSE prior to *S. haematobium* egg injection alters urothelial inflammation. Together, these results suggest that *S. haematobium* IPSE also functions as an immunomodulatory protein, and plays an important and distinct role in regulating tissue pathology in urogenital schistosomiasis.

1793

GENDER DEPENDENCE OF P53-RELATED ABNORMALITIES IN MOUSE BLADDER UROTHELIUM DUE TO *SCHISTOSOMA HAEMATOBIUM* EGG-INDUCED INFLAMMATION

Jared Honeycutt[1], Olfat Hammam[2], Michael H. Hsieh[1]

[1]Stanford University School of Medicine, Stanford, CA, United States, [2]Theodor Bilharz Research Institute, Cairo, Egypt

The bladder urothelium is dramatically altered during *Schistosoma haematobium* infection (urogenital schistosomiasis). These alterations include hyperplasia, ulceration, dysplasia, squamous metaplasia and frank carcinogenesis. Defining the pathways that drive these urothelial changes will contribute to a deeper understanding of how *S. haematobium* egg-induced expulsion, hematuria, and bladder cancer develop in humans. Defects in the function of the tumor suppressor gene p53 are evident in many cancers, including bladder cancer generally and schistosomal bladder cancer specifically. To identify any role p53 might play in urothelial alterations due to urogenital schistosomiasis, we employed transgenic mice with tamoxifen-inducible cre recombinase activity in cells expressing

uroplakin-3a, a urothelial-specific gene (Upk3a-GCE mice). We confirmed specificity of cre expression in Upk3a-GCE mice by crossing them with TdTomato-floxed-EGFP reporter mice and administering tamoxifen to their progeny. As expected, these progeny switched from TdTomato to EGFP expression in their bladder urothelium. We then crossed Upk3a-GCE mice to p53-floxed mice. The resulting progeny (Upk3a-GCE+/wt;p53fl/wt) were given tamoxifen or vehicle control to render them urothelial p53-haploinsufficient or -intact, respectively. We then injected *S. haematobium* eggs or control vehicle into the bladder walls of these mice. Three months later, mice were sacrificed and their bladders subjected to histological analysis (H&E staining). Male p53-intact, egg-injected mice exhibited similar histological changes as their p53-haploinsufficient counterparts, including urothelial hyperplasia and ulceration. In contrast, female p53-intact, egg-injected mice featured no urothelial ulceration, whereas their p53-haploinsufficient counterparts often had significant ulceration. Additionally, some egg-injected p53-haploinsufficient females exhibited regions of squamous metaplasia. Thus, intact p53 activity seems to be required, in a gender-specific manner, for urothelial homeostasis during *S. haematobium* infection in this model. Ongoing work includes (1) examining histological changes in Upk3a-GCE+/wt;p53fl/wt mice beyond 3 months after egg-injection, and (2) measuring alterations in the cell cycle status of the urothelium as a consequence of schistosomiasis.

1794

CD4+ T-CELL COUNTS IN WOMEN WITH UROGENITAL SCHISTOSOMIASIS

Elisabeth Kleppa[1], Kari F. Klinge[1], Kristine Lillebø[1], Hashini N. Galaphaththi-Arachchige[1], Sigve D. Holmen[1], Siphosenkosi G. Zulu[2], Birgitte J. Vennervald[3], Pavitra Pillay[2], Mathias Onsrud[1], Svein Gunnar Gundersen[4], Myra Taylor[2], Eyrun F. Kjetland[1]

[1]Oslo University Hospital, Oslo, Norway, [2]University of KwaZulu-Natal, Durban, South Africa, [3]University of Copenhagen, Copenhagen, Denmark, [4]Sorlandet Hospital, Kristiansand, Norway

Schistosoma haematobium causes urogenital schistosomiasis and has been shown to be associated with HIV in cross-sectional studies. Schistosomiasis infection has been hypothesized to affect the HIV viral load and HIV disease progression. Schistosomiasis treatment has been shown to increase CD4 counts in HIV negative individuals. Between May and October 2013, a CD4 count was done in 797 young women who were invited for a gynecological examination. One urine sample was collected from all, and microscopy for schistosome ova was done. HIV testing was done in 769 women, of which 123 were HIV positive (16.0%). The mean CD4 count was 864 x10^6 cells / L. It was lower in the HIV negative group than in the HIV positive group (931 vs. 511 $p < 0.001$). Urinary schistosomiasis was not associated with a lower CD4 count (862 in urine negative vs. 871 in urine positive women, p=0.75). Likewise, there was no significant association between CD4 counts between women with moderate or severe degree genital schistosomiasis and women without genital lesions (846 vs. 829, respectively, p= 0.70). Further there was no significant difference between schistosomiasis positive and negative women were found after stratifying for HIV (data not shown). This cross-sectional study did not show any significant difference in CD4 T-cell counts when comparing women with and without urogenital schistosomiasis.

1795

PATTERNS OF REACTIVITY TO *SCHISTOSOMA MANSONI* EGG GLYCAN ANTIGENS IN A POPULATION OF TREATMENT-NAÏVE KENYAN SCHOOL CHILDREN

Nina S. Prasanphanich[1], Jasmine A. Landry[2], Ryan E. Wiegand[3], Eric M. Ndombi[4], Bernard Abudho[4], Diana K. Riner[5], Megan L. Mickum[1], Diana M. Karanja[4], Daniel G. Colley[5], Richard D. Cummings[1], W. Evan Secor[3]

[1]Emory University, Atlanta, GA, United States, [2]Macalester College, St. Paul, MN, United States, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States, [4]Kenya Medical Research Institute, Kisumu, Kenya, [5]University of Georgia, Athens, GA, United States

Children in schistosomiasis-endemic areas develop partial resistance to infection as they age. This resistance is associated with immune responses, including IgE and IgG, to parasite antigens. Anti-glycan antibodies can kill parasite larvae *in vitro* and mediate resistance in some animal models of helminth infection, but their significance in human *Schistosoma mansoni* infection is still unclear. Plasma from *S. mansoni*-infected children demonstrate specific reactivity with several epitopes on schistosome glycan microarrays. To explore the relationship of such antibodies with naturally-acquired partial immunity, we measured IgG and IgM to mock- and periodate-treated *S. mansoni* soluble egg antigen (SEA), and two parasite cross-reactive glycoproteins, keyhole limpet hemocyanin (KLH) and horseradish peroxidase (HRP), in plasma from a population of treatment-naïve Kenyan school children. The ratio of antibody reactivity with periodate-resistant (primarily non-glycan) versus total epitopes in SEA increased, and antibodies to KLH and HRP glycans decreased slightly as children aged. These trends were especially pronounced throughout adolescence. The anti-glycan antibodies detected included a variety of IgG subtypes. Our results suggest that immune recognition of the glycan epitopes examined in this study are negatively associated with age, but some may warrant further investigation as diagnostics or indicators of the length of exposure to schistosomes. Future studies on anti-glycan antibodies to other epitopes or of other isotypes/subtypes in naturally-acquired immunity, and on whether anti-glycan antibodies may be involved in resistance to reinfection after praziquantel treatment could be informative for vaccine development.

1796

CHILDREN WITH CEREBRAL MALARIA LACK SERORECOGNITION OF A DISTINCT PFEMP1 SUBSET

Mark A. Travassos[1], Amadou Niangaly[2], Jason A. Bailey[1], Amed Ouattara[1], Drissa Coulibaly[2], Kirsten Lyke[3], Matthew B. Laurens[1], Jozelyn Pablo[4], Algis Jasinskas[4], Rie Nakajima-Sasaki[4], Andrea A. Berry[1], Matthew Adams[1], Shannon Takala-Harrison[3], Bourema Kouriba[2], Abdoulaye K. Kone[2], J. Alexandra Rowe[5], JoAnn Moulds[6], Ogobara K. Doumbo[2], Mahamadou A. Thera[2], Philip L. Felgner[4], Christopher V. Plowe[1]

[1]Howard Hughes Medical Institute/Center for Vaccine Development, Baltimore, MD, United States, [2]Malaria Research and Training Center, Bamako, Mali, [3]Center for Vaccine Development, Baltimore, MD, United States, [4]University of California Irvine, Irvine, CA, United States, [5]University of Edinburgh, Edinburgh, United Kingdom, [6]LifeShare Blood Centers, Shreveport, LA, United States

Plasmodium falciparum erythrocyte membrane protein-1 (PfEMP1) antigens play an important role in parasite sequestration and host immune system evasion. Acquired antimalarial immunity is at least partially due to antibodies directed against highly variable antigens like PfEMP1 that are present on the red blood cell surface. However, the PfEMP1 antigenic domains that drive this immune-mediated protection have not been identified. We have previously shown that infected erythrocytes from persons with cerebral malaria express a distinct "stealth" PfEMP1 group that do not bind the endothelial receptor CD36. We hypothesized that children with cerebral malaria lack serorecognition to a subset of PfEMP1s

that is subsequently recognized in convalescence. A protein microarray was printed with 171 fragments of PfEMP1s based on the 3D7 reference genome. For comparison, 268 diverse apical membrane 1 (AMA1) fragments, 20 merozoite surface protein 1 (MSP1) fragments, and 30 Rh5 fragments were also included on the array, based on sequences derived from field samples. Reactivity was measured in 195 serum samples from Malian children, including 43 cases of cerebral malaria and age-matched controls who were healthy or had uncomplicated malaria. Children with cerebral malaria had lower seroreactivity to stealth and non-stealth PfEMP1 antigen variants than both healthy controls and uncomplicated malaria controls. Seroreactivity to AMA1, Rh5, and MSP1 variants tested did not increase from acute cerebral malaria illness to convalescence, but seroreactivity to four stealth PfEMP1 fragments increased, suggesting that a lack of immunity to a subset of PfEMP1s may be associated with vulnerability to cerebral malaria.

1797

CYTOKINE RESPONSES TO THE VAR2CSA VACCINE CANDIDATE IN CORD BLOOD FROM BENINESE NEWBORNS

Nadine Fievet[1], Komi Gbedandé[2], Gilles Cottrell[1], Bertin Vianou[2], Carine Agbowaï[2], Samad Ibitokou[2], Marita Troye-Blomberg[3], Thor Theander[4], Ali Salanti[4], Achille Massougbodji[2], Nicaise Tuikue Ndam[1], Philippe Deloron[1], Adrian Luty[1]

[1]IRD UMR216 "Mère et enfant face aux infections tropicales", Paris, France, [2]Centre d'Etude et de Recherche sur le Paludisme Associé à la Grossesse et à l'Enfance, Faculté des Sciences de la Santé, Cotonou, Benin, [3]Department of Immunology, Wenner-Gren Institute, Stockholm University, Stockholm, Sweden, [4]Department of International Health, Institute of International Health, Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark

The STOPPAM consortium conducted 2 longitudinal cohort studies in pregnant women in Benin and Tanzania in order to evaluate the immunopathological consequences of infections with *Plasmodium falciparum* during pregnancy for the newborns. In order to study the exposure of the foetal immune system to parasite-derived antigens *in utero*, we evaluated both, cytokine (IL10, IL12, IL13, IL17, IFN-γ, TNF-α responses and T cell IFN-γ specific responses to the vaccine candidate antigen DBL5 domain of VAR2CSA as a function of placental infection with *P. falciparum*. In Come, southwestern Benin, we conducted a longitudinal prospective study of ~1000 pregnant women. Women at ≤24 weeks of pregnancy were enrolled and followed at each antenatal visit until delivery. For the immunological sub-study of the cord blood mononuclear cellular (CBMC) responses to VAR2CSA-DBL5 *in vitro*, a group of 200 pregnant women was selected at delivery on the basis of their history of infection with *P. falciparum* (uninfected during pregnancy/infected during pregnancy but uninfected at delivery/infected at delivery). Those harbouring *P. falciparum* infections at delivery were matched by gravidity and gestational age with mothers with no infection and those with no history of infection earlier in the pregnancy. The amounts of IL10, IL12, IL13, IL17, IFN-γ and TNF-α produced in response to mitogen (PHA) and to VAR2CSA-DBL5 were quantified in supernatants of stimulated CBMC. The *ex vivo* frequencies of IFN-γ secreting CD4 and CD8 T cells in response to PHA and VAR2CSA domains were also evaluated. At the time of writing, all data have been collected, cytokine concentrations have been evaluated and multivariate analyses are under way. Results will be discussed in the context of cytokine profiles that reflect the *in utero* acquisition of a specific cellular memory response to the vaccine candidate.

1798

RNA-SEQ ANALYSIS OF WHOLE BLOOD FROM MALARIA-SUSCEPTIBLE AND IMMUNE CHILDREN REVEALS AN EARLY PRO-INFLAMMATORY RESPONSE TO *PLASMODIUM FALCIPARUM* INFECTION THAT CORRELATES WITH CONTROL OF PARASITE GROWTH: A PROSPECTIVE STUDY IN MALI

Tuan M. Tran[1], Aissata Ongoiba[2], Marcus Jones[3], Jeff Skinner[1], Shanping Li[1], Safiatou Doumbo[2], Didier Doumtabe[2], Younoussou Kone[2], Aboudramane Bathily[2], Seydou Dia[2], Moussa Niangaly[2], Abdrahamane Traore[2], Charles Dara[2], Jules Sangala[2], Ogobara K. Doumbo[2], Pratap Venepally[3], Kassoum Kayentao[2], Boubacar Traore[2], Ewen F. Kirkness[3], Peter D. Crompton[1]

[1]Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [2]Mali International Center of Excellence in Research, University of Sciences, Technique and Technology of Bamako, Bamako, Mali, [3]Genomic Medicine Group, J. Craig Venter Institute, Rockville, MD, United States

Non-sterile, antibody-mediated immunity that reliably protects from febrile malaria is acquired gradually through repeated *Plasmodium falciparum* infections; however, the nature of cellular immune responses at the onset of clinically apparent versus clinically silent blood-stage infections in children is unclear. In a prospective study in Mali, we collected whole blood RNA, PBMCs and plasma from healthy, uninfected children aged 6-11 years (n=79) before the 6-month malaria season and from the same children during their first *P. falciparum* infection of the ensuing season—detected retrospectively through bi-weekly active surveillance by PCR. We used RNA-seq to compare whole-blood transcriptomes of children whose clinically silent infections never progressed to fever (immune, n=21), children whose infections progressed to fever within 2-14 days (late fever, n=32) and children who were febrile at the time of infection (early fever, n=26). We found that baseline transcription profiles before the malaria season distinguished children whose future *P. falciparum* infections either progressed to fever or not, including upregulation of B-cell-receptor signaling pathways in immune children. Transcription profiles induced by the first detected *P. falciparum* infection of the season revealed upregulation of pro-inflammatory genes in immune versus late fever children, despite both groups having similar levels of parasitemia and the clinical absence of fever initially. In addition, this early upregulation of pro-inflammatory genes was associated with slower subsequent parasite growth rates *in vivo*. In ongoing work, we are testing hypotheses generated by this study at the protein level and in functional assays using contemporaneous PBMCs and plasma samples from the same children. Molecular and cellular signatures that correlate with protection from malaria are yielding novel insights into the mechanisms underlying naturally acquired immunity to malaria. The resulting datasets may inform the development of interventions that prevent or mitigate malaria disease.

1799

IMMUNE CHARACTERIZATION OF *PLASMODIUM FALCIPARUM* PARASITES WITH A SHARED GENETIC SIGNATURE: VARIANT SURFACE ANTIGENS AND *VAR* REPERTOIRES

Amy K. Bei[1], Ababacar Diouf[2], Kazutoyo Miura[2], Daniel B. Larremore[3], Ulf Ribacke[1], Gregory Tullo[2], Eli L. Moss[4], Daniel E. Neafsey[4], Rachel F. Daniels[1], Amir E. Zeituni[2], Iguosadolo Nosamiefan[1], Sarah K. Volkman[1], Ambroise D. Ahouidi[5], Daouda Ndiaye[6], Tandakha Dieye[5], Souleymane Mboup[5], Caroline O. Buckee[3], Carole A. Long[2], Dyann F. Wirth[1]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, [3]Harvard School of Public Health, Center for Communicable Disease Dynamics, Boston, MA, United States, [4]Broad Institute of Massachusetts Institute of Technology and Harvard, Boston, MA, United States, [5]Laboratory of Bacteriology and Virology, Hopital Aristide Le Dantec, Dakar, Senegal, [6]Laboratory of Parasitology and Mycology, Dakar, Senegal

As malaria transmission intensity has declined in some regions, *Plasmodium falciparum* parasite populations are displaying decreased clonal diversity resulting from the emergence of many parasites with identical genetic signatures. We have monitored genetically identical parasite clusters from 2006-2013 in Thiès, Senegal, and we have characterized the immune response against these parasites. We focus on one cluster of identical parasites that was present in 24% of clinical isolates in 2008 and declined to 3.4% of clinical isolates in 2009. We studied the susceptibility of 2 representative common genetic signature (CGS) parasites and 1 representative non-CGS parasite and measured the infected RBC IgG reactivity for 109 individual plasmas distributed between both years by variant surface antigen (VSA) flow cytometry. By VSA flow, the non-CGS parasites are similary recognized by plasma IgG from 2008 and 2009, but reactivity is increased in 2009 compared to 2008 for the CGS parasites. We characterized the *var* genes expressed by CGS parasites by *var* Ups qRT-PCR and by sequencing using degenerate DBL1alpha domain primers. We observed that the CGS parasites expressed the same *var* Ups classes, and the same dominant *var* repertoires as identified by both DBL1alpha sequence analysis as well as RNAseq. Additionally, we used network analysis to compare the diversity of the *var* repertoires with that of globally diverse parasites. We generated a *var* sequence network that shows that the *var* repertoires of CGS-1 and CGS-2 overlap substantially, while the repertoire of the non-CGS parasite is unique at the level of globally diverse parasites. Taken together, our work indicates that these CGS parasites express similar *var* genes, more than would be expected by chance in the population, and there is year-to-year variation in immune recognition of these CGS parasites at the level of surface expression of VSAs. We are currently expanding these findings to other large clusters such as one that emerged in 2009 and persisted at a high frequency (26-30%) for multiple years before disappearing from the population in 2013.

1800

SINGLE-CELL TRANSCRIPTIONAL ANALYSIS OF MALARIA-SPECIFIC CD4+ T LYMPHOCYTES FOLLOWING PFSPZ VACCINATION AND PROTECTION IN HUMANS

Kailan Sierra-Davidson[1], Adam Wheatley[1], Patricia Darrah[1], Greg Finak[2], Madhu Prabhakaran[1], Pratip Chattopadhyay[1], Mario Roederer[1], Helen McShane[3], Kim Lee Sim[4], Stephen Hoffman[4], Raphael Gottardo[2], Adrian McDermott[1], Robert Seder[1]

[1]National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, [2]Vaccine and Infectious Disease Division, Seattle, WA, United States, [3]Jenner Institute, Oxford, United Kingdom, [4]Sanaria, Rockville, MD, United States

Intravenous immunization with highly purified, radiation-attenuated parasites (PfSPZ Vaccine) is safe, immunogenic and confers high-level protection against controlled malaria infection in humans. Protection was associated with a dose-dependent increase in PfSPZ-specific antibodies, $CD4^+$ and $CD8^+$ T cell responses. Heretofore, multi-parameter flow cytometry has been used to characterize the magnitude and quality of PfSPZ-specific T cell responses following vaccination or infection. To substantially expand the analysis of such responses, we performed high-resolution, quantitative transcriptome analysis of PfSPZ-specific $CD4^+$ T cells. Accordingly, PfSPZ-specific $CD4^+$ T cells expressing the costimulatory marker CD154 (CD40L) were sorted following *in vitro* activation with sporozoites. We first demonstrate that ~30 percent of PfSPZ-specific $CD154^+$ $CD4^+$ T cells do not produce IFN-γ, IL-2 or TNFα, the most common cytokines used to assess T cell responses. This finding highlights the increased sensitivity of the CD154 capture assay for broader assessment of antigen-specific responses. Furthermore, isolation of live malaria-specific $CD4^+$ T cells permits downstream mRNA analysis using valved microfluidic chips from Fluidigm. Quantitative expression of ~100 genes can be rapidly analyzed from isolated samples of single antigen-specific T cells. Initial transcriptome analysis of protected subjects revealed that malaria-specific $CD4^+$ T cells express a unique gene expression signature that is distinct from influenza-specific $CD4^+$ T cells in the same individual. These data will serve as an internal control to compare virus- and parasite-specific responses. We are currently analyzing the gene signature of PfSPZ-specific $CD4^+$ T cells from vaccinated and protected subjects prior to challenge vs. nonvaccinated controls during infection. Overall, this analysis should advance our understanding of the heterogeneity of parasite-specific $CD4^+$ T cell responses at the single-cell level and provide insights into how $CD4^+$ T cells may influence protection against human malaria infection.

1801

IMMUNOLOGICAL PROFILING AFTER SPOROZOITE IMMUNIZATION UNDER CHEMOPROPHYLAXIS IN THE CONTROLED HUMAN MALARIA INFECTION MODEL

Robert W. Sauerwein

Radboud University Medical Center, Nijmegen, Netherlands

A unique tool to study malaria immunology and efficacy of immunisation strategies form Controlled Human Malaria Infections (CHMI) and has proved to be a reproducible, predictable and safe method of inducing *Plasmodium falciparum* (Pf) malaria. An efficient method for induction of complete protection in humans was achieved by exposing human subjects to Pf-infected mosquitoes while taking blood-stage suppressive chloroquine prophylaxis. When tested in clinical trials, this protocol induced > 95% clinically and parasitologically sterile protection against a standard challenge infection. Longlasting CPS-induced protection was primarily mediated by immunity to sporozoite and liver stages rather than to asexual blood-stages. This opens opportunities to explore mechanisms of protective immunity, allowing the search for immune correlates/signatures of protection and clinical development of a whole sporozoite based vaccine. Humoral and cellular immune responses associated with protection to *Plasmodium falciparum* parasites will be presented.

1802

GENE PROFILING IN NAÏVE AND SEMI-IMMUNE COLOMBIAN INDIVIDUALS SUBJECTED TO EXPERIMENTAL CHALLENGE WITH *PLASMODIUM VIVAX* SPOROZOITES

Myriam Arévalo-Herrera[1], Monica Rojas-Peña[2], Andres F. Vallejo[1], Nora Martinez[1], Greg Gibson[2], Sócrates Herrera[1]

[1]Caucaseco Scientific Research Center, Cali, Colombia, [2]School of Biology, Georgia Institute of Technology, Atlanta, GA, United States

In the context of a *Plasmodium vivax* malaria vaccine program, a total of 16 Colombian malaria naïve (n=7 from Cali) and semi-immune (n=9 from Buenaventura) volunteers were subjected to an experimental P.vivax sporozoite infectious challenge using direct infected *Anopheles* mosquito bites (2-4) and followed up to determine the prepatent period, immune response and clinical outcome of the infection. Volunteers were closely monitored and treated as soon as malaria infection was detected by microscopy. The study offered a unique opportunity to assess the gene expression profile induced by *P. vivax* malaria infection in naïve and previously infected human volunteers Blood samples were used for immunological analyses and RNA preserved in Tempus tubes was isolated for transcriptomic analyses. We used a Fluidigm nanofluidic qRT-PCR array to profile the expression of 92 genes in the 16 individuals across 6 time-points following infection. The genes were chosen to represent 10 axes of variation that describe major components of transcriptional variation in peripheral blood. Strong covariance of transcript abundance was observed for 8 of these axes, 2 of which correspond to the first two overall principal components of variation. The results show that there is strong up-regulation of an interferon-response axis at the peak of parasitemia, but a down-regulation of the inflammatory response at the same time. Another set of transcripts was observed to be significantly up-regulated both in the naïve samples and at the peak of parasitemia, but across the entire experiment the difference between the naïve and pre-immune samples was minor relative to among individual variation. Nevertheless, these results strongly suggest that whole transcriptome profiling will uncover a set of genes that respond differently to infection as a function of the degree of prior exposure to malaria..

1803

GAMETOCYTE CLEARANCE IN MELANESIAN CHILDREN TREATED FOR *PLASMODIUM FALCIPARUM* AND *P. VIVAX* MALARIA WITH ARTEMISININ COMBINATION THERAPY

Stephan Karl[1], Moses Laman[2], Tamarah Koleala[3], Anna Rosannas-Urgell[4], Brioni Moore[2], Peter Siba[3], Timothy G. St.Pierre[2], Ivo Mueller[1], Timothy Davis[2]

[1]Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, [2]The University of Western Australia, Perth, Australia, [3]Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea, [4]Antwerp Institute of Medical Research, Antwerp, Belgium

We present a detailed analysis of gametocyte clearance in Melanesian children infected with *Plasmodium falciparum* or *P. vivax* and treated with either Artemether-Lumefantrine (AL) or Artemisinin-Naphthoquine (AN). In addition, a detailed comparison of three methods, namely standard light microscopy (LM), magnetic fractionation (MF) and reverse transcriptase polymerase chain reaction (RTPCR) for detection of *Plasmodium falciparum* and *Plasmodium vivax* gametocytes is presented. Children (0.5-5 years) from the north coast of Papua New Guinea were randomly assigned to either AL or AN treatment upon presentation at the health centre with either *P. falciparum* or *P. vivax* malaria. LM was conducted by 2 trained microscopists with discordant reads judged by an expert microscopist. MF was conducted as previously described on the same day using 200 µL of blood. Samples for RTPCR were placed directly into RNA-later and stored at -80°C until analysis. MF and RTPCR were similarly sensitive and specific, and clearly superior to LM detection of gametocytes. *P. falciparum* gametocyte clearance characteristics were found to be different between AL and AN, mostly due to a longer predicted gametocyte sequestration time in the AN group. However AN treatment provided longer protection from gametocytaemic relapse and/or reinfection. *P. vivax* gametocytes were found to be cleared very rapidly and along with the asexual blood stages upon treatment with AL or AN, highlighting the fundamental differences between the *P. falciparum* and the *P. vivax* parasite species. This study represents the first direct comparison of LM, MF and RTPCR on a large number of field isolates. It provides clear evidence that magnetic fractionation is superior to light microscopy and can be used to detect gametocytaemic patients under field conditions with similar sensitivity and specificity as RTPCR. Furthermore this study illustrates fundamental differences between ACT mediated clearing of *P. falciparum* and P.vivax gametocytes and describes differences in the effect of AL and AN on *P. falciparum* gametocytes.

1804

DEVELOPMENT AND EVALUATION OF A SIMPLIFIED MOLECULAR DIAGNOSTIC PLATFORM FOR MALARIA: THE DIRECT ON BLOOD PCR-NALFIA SYSTEM

Petra Mens, Johanna Roth, Laura de Bes, Henk Schallig

Royal Tropical Institute, Amsterdam, Netherlands

Molecular tools allow for specific and sensitive malaria diagnosis, but current formats, like PCR with gel-electrophoresis, are difficult to implement in resource poor settings. Therefore, a simple, fast, sensitive and specific molecular diagnostic platform, direct on blood (db)PCR combined with nucleic acid lateral flow immunoassay (NALFIA) to detect amplified PCR products of *Plasmodium*, including species differentiation, and human GAPDH (internal amplification control) was developed. This platform does not require DNA extraction and circumvents complex read-out system. The platforms was evaluated under laboratory conditions, a multi country ring trial and in two malaria endemic countries (Burkina Faso and Thailand). Analytical sensitivity and specificity of the dbPCR-NALFIA in a single laboratory evaluation was >95% and the test was able to detect less than 1 parasite/µl blood. All four laboratories in the ring trial reported ease of use of the system and could successfully perform the protocol. Overall laboratory inter-variability was low and the agreement of reported results was high. Overall k-value was 0.89 (95% CI: 0.83 - 0.94; $p<0.001$). Overall test sensitivity and specificity was >95% with very small confidence intervals. Field evaluations by local staff without prior training in performing the dbPCR-NALFIA in malaria endemic countries, Thailand and Burkina Faso, were performed. In Burkina Faso (*P. falciparum* environment) the relative sensitivity was 94,8% and relative specificity 82,4% compared to microscopy and 93,3% and 91.4% compared to RDT. In Thailand (*P. vivax* environment) the relative sensitivity and relative specificity was 93,4% and 90,9 respectively compared to microscopy and 95,6% and 87.1 % compared to RDT. These numbers are an underestimation of test performance as the results are not PCR corrected. The prototype dbPCR-NALFIA test will now be moved forward in diagnostic test development (supported by EU funding: www.diagmal.eu) to provide a molecular diagnostic test to detect malaria in for example near elimination settings. The final format will include a closed transfer unit to reduce possible workspace contamination with amplicons. Funding: EU FP7 grant 601714: Translation of the direct-on-blood PCR-NALFIA system into an innovative near point-of-care diagnostic for malaria

1805

DEVELOPMENT OF A SINGLE NUCLEOTIDE POLYMORPHISM BASED BARCODE FOR THE IDENTIFICATION AND TRACKING OF *PLASMODIUM VIVAX*

Mary Lynn Baniecki[1], Aubrey Faust[2], Rachel Daniels[3], Kevin Galinsky[4], Marcelo U. Ferreira[5], Nadira Karunaweera[6], Elizabeth Winzeler[7], David Serre[8], Peter Zimmerman[9], Tom Wellems[10], Lise Musset[11], Stéphane Pelleau[11], Alexandre Melnikov[1], Daniel Neafsey[1], Sarah Volkman[4], Daniel L. Hartl[2], Dyann Wirth[4], Pardis Sabeti[2]

[1]The Broad Institute, Cambridge, MA, United States, [2]Harvard University, Cambridge, MA, United States, [3]Harvard School of Public Health, Cambridge, MA, United States, [4]Harvard School of Public Health, Boston, MA, United States, [5]University of São Paulo, São Paulo, Brazil, [6]University of Colombo, Colombo, Sri Lanka, [7]The University of California San Diego, San Diego, CA, United States, [8]Cleveland Clinic, Cleveland, OH, United States, [9]Case Western Reserve University, Cleveland, OH, United States, [10]National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, [11]Institut Pasteur de la Guyane, Cayenne, French Guiana

For global eradication of malaria to occur an enhanced understanding of the population structure of *Plasmodium vivax* is needed. An inability to maintain *P. vivax* continuously in culture has caused research on this parasite to lag behind that of the other *Plasmodium* species. Recent advances in molecular methods have enabled the *P. vivax* genome to be assayed directly from infected humans, providing the tools needed to develop genotyping methods. Single nucleotide polymorphism (SNP) genotyping provides a robust, inexpensive, field-deployable technology that allows malaria parasites to be tracked and identified by creating a unique genetic signature or barcode for each parasite. Using our experience in developing a SNP genotyping tool with quantitative PCR and High Resolution Melting (qPCR-HRM) for *P. falciparum*, we have developed a SNP barcode for *P. vivax.* The candidate SNPs were selected with a high minor allele frequency (MAF) from available *P. vivax* genome sequence data and were located at sites including intergenic, intragenic, or were 4-fold degenerate coding sites. Here, we report a pilot screen of a 95 SNPs by genotyping a set of 89 *P. vivax* containing clinical samples from geographically distinct parasite populations from the Americas (Brazil, French Guiana), Africa (Ethiopia) and Asia (Sri Lanka). Candidate SNPs were winnowed to a 41-SNP barcode based on robustness and reproducibility of the genotyping calls, and the ability to accurately detect polygenomic infections. The assays are robust with a detection range from 10 ng to 0.001 ng with an average assay efficiency of 90% among clinical samples tested. All 41 assays had an average minor allele frequency (MAF) > 0.1. Based on principle component analysis the clinical samples form distinct clusters that correspond to their geographic origin. Interesting, these analysis revealed a high level of polygenomic samples among all populations with Brazil (84%), Sri Lanka (95%), Ethiopia (78%), and French Guiana (62%).

1806

THE APICOPLAST OF *PLASMODIUM FALCIPARUM* PROVIDES A NOVEL TARGET FOR MOLECULAR DIAGNOSIS OF MALARIA USING POLYMERASE CHAIN REACTION AND LOOP MEDIATED ISOTHERMAL AMPLIFICATION ASSAYS

Eniyou C. Oriero[1], Jan Jacobs[2], Jean-Pierre Vangeertruyden[3], Umberto D'Alessandro[1], Davis Nwakanma[1]

[1]Medical Research Council Unit, The Gambia, Fajara, Gambia, [2]Institute of Tropical Medicine, Antwerpen, Belgium, [3]University of Antwerp, Antwerpen, Belgium

Despite a recent increase in procured rapid diagnostic tests (RDTs), and rate of diagnostic testing in the public sector of the African region, malaria still remains a global health burden with an estimated 627,000 deaths worldwide in 2012. WHO recommends all suspected malaria cases to be confirmed by microscopy or RDT prior to treatment however, millions of people with suspected malaria still do not receive diagnostic tests. As these methods do not rapidly and accurately detect sub-microscopic infections which can also contribute to transmission, high throughput molecular assays such as polymerase chain reaction (PCR) are used to detect asymptomatic and/or low-grade infections. Isothermal amplification methods were recently developed to address some of the major shortcomings of PCR. The most common target amplified in these molecular assays is the conserved small subunit ribosomal RNA 18S locus, which in the *Plasmodium falciparum* chromosomal genome exists in five to eight copies, depending on the strain. In this study, we report the development and optimization of the apicoplast of *P. falciparum* as a target for molecular diagnosis of malaria using a single step PCR (ssPCR), nested PCR (nPCR) and loop-mediated isothermal amplification (LAMP) assay. *P. falciparum* sequences from 15 Gambian isolates and 8 laboratory clones were aligned against the PlasmoDB reference sequence (ID: emb|X95275.2|) and primers were designed from a highly conserved region of the consensus sequence, approx. 1.5kb segment of the gene coding for a ribosomal RNA protein (AP|0010:rRNA). The primers were validated in silico and mapped unto the consensus sequence with a web based tool. The assays were optimized for temperature and concentration of primers, deoxyribonucleotides (dNTPs) and magnesium chloride (MgCl2). 272 archived DNA samples from across West Africa and S.E Asia were analyzed against a reference PCR method targeting the 18SrRNA gene. Preliminary results show perfect agreement for ssPCR and nPCR compared with the reference PCR method, while Sensitivity of 100 % (95% CI: 94 % to 100 %), Specificity of 84 % (95% CI: 68 % to 94 %), Positive Predictive Value (PPV) of 91 % (95% CI: 81 % to 97 %), Negative Predictive Value (NPV) of 100 % (95% CI: 89 % to 100 %) and Kappa index of 0.86 (95% CI: 0.76 to 0.97) were obtained for LAMP. Based on the results, the apicoplast genome appears to be a suitable target for sensitive detection of P.falciparum.

1807

GEOGRAPHICAL DISPERSION AND GENETIC CHARACTERIZATION OF PFHRP2 NEGATIVE *PLASMODIUM FALCIPARUM* PARASITES IN THE PERUVIAN AMAZON: IMPLICATIONS FOR RAPID DIAGNOSTIC TESTS (RDTS) BASED ON DETECTION OF HRP2

Dionicia Gamboa[1], Jorge Bendezu[1], Katherine Torres[1], Sheila Akinyi[2], Curtis S. Huber[2], Elizabeth Villasis[1], Sandra Incardona[3], David Bell[3], Joseph Vinetz[4], John W. Barnwell[2], Venkatachalam Udhayakumar[2]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Malaria Branch, Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Foundation for Innovative New Diagnostics, Geneva, Switzerland, [4]University of California San Diego, San Diego, CA, United States

There is prior evidence of *Plasmodium falciparum* parasites from clinical cases lacking pfhrp2 and pfhrp3 genes in the Peruvian Amazon Region. As various countries in South America move to introduce malaria rapid diagnostic tests (RDTs) as an alternative for diagnosis, the geographical distribution and genotypic characterization of parasites lacking pfhrp2 and pfhrp3 genes will have major implications for procurement choices for RDTs in the region. Ninety-three *P. falciparum* samples, collected in different communities from the Peruvian Amazon Region between 2009 and 2010, were used in this study. Genomic DNA was used to amplify 18SrRNA and pfmsp2 to confirm the diagnosis and DNA quality, respectively; pfhrp2, pfhrp3, and their flanking genes in order to assess the frequency of their deletions. Microsatellite analysis was performed using seven neutral microsatellites (MS) and seven novel MS loci flanking the pfhrp2 gene on chromosome 8 (-41kb, -10kb, -4kb, 1.4kb, 2.5kb, 5.2kb and 15kb). The data showed deletion of the pfhrp3 gene in 53.76% (50/93) and pfhrp2 gene deletion in 33.33% (31/93) of the samples. The proportion of the parasite populations that lacked these genes was quite variable from community to community. Among the flanking genes, PF3D7_0831900 (Mal7P1.230) showed the highest deletion frequency,

78.49% (73/93). Neutral MS marker analysis revealed the widespread distribution of *P. falciparum* hybrid lineages with a hybrid of the A clonal lineage (named AV1) being the most prevalent among parasites lacking pfhrp2 and pfhrp3 genes. MS data from loci flanking the pfhrp2 gene showed that the haplotypes α and Δ were the most abundant among the isolates analyzed. This study confirms that field isolates lacking either pfhrp2, pfhrp3 or their respective flanking genes were still present in the area in 2010. In addition, we identified five *P. falciparum* hybrid lineages circulating in this region. It is possible that certain parasite genetic backgrounds (haplotypes) could favor the maintenance and expansion of pfhrp2 and pfhrp3 gene deletions in the Peruvian Amazon, however further studies will be required to prove this possibility and also to elucidate the genetic basis for the pfhrp2 gene deletion in wild *P. falciparum* parasites.

1808

AN EXTERNAL QUALITY ASSURANCE PROTOCOL FOR PLACENTAL MALARIA HISTOPATHOLOGY STUDIES

Atis Muehlenbachs[1], Miriam K. Laufer[2], Tamiwe Tamoka[3], Veronica Ades[4], Grant Dorsey[5], Julie Gutman[6]

[1]Infectious Diseases Pathology Branch, Division of High Consequence Pathogens and Pathology, Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Department of Pediatrics, University of Maryland, Baltimore, MD, United States, [3]University of Malawi College of Medicine, Blantyre, Malawi, [4]Department of Obstetrics and Gynecology, New York University Langone Medical Center, New York, NY, United States, [5]Department of Medicine, University of California San Francisco, San Francisco, CA, United States, [6]Malaria Branch, Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, GA, United States

Placental histology can contribute to studies on the diagnosis, prevention and treatment of malaria in pregnancy (MIP). Infections prior to delivery can be identified by detecting hemozoin deposition, and parasitized erythrocytes can be seen in cases where peripheral blood is negative. However histology is prone to artifacts, and requires considerable training in slide reading. There is a general need for external quality control and quality assurance for MIP histopathology studies, where poor preparation can lead to the appearance of a hemozoin-like material which may be mistaken for evidence of prior malaria infection and debris can be mistaken for parasites, leading to false positive reads. In addition, very low level infections may be present in the placentas of semi-immune women, leading to false negatives if insufficient fields are examined or if the slide reader is inexperienced. We developed a protocol to formally provide confidential, blinded, retrospective review of slides with feedback in order to ensure high quality study data and to work towards building local expertise. A random subset of 10% of negative and 25% of positive slides are recommended for review by a single, blinded expert reader. Slides are formally scored on both the presence of parasites and hemozoin in fibrin in addition to general quality. Following unblinding, discrepant slides are re-examined to determine source of discrepancy, and consensus opinion with the submitter is attempted by using high quality photomicrographs. All slides are returned to the submitter so that they can be used for further education. Error rates less than 20% are considered standard for passing a laboratory proficiency test, and further rounds of QC can be performed as needed. For histology studies of malaria in pregnancy, discordance over 10% should trigger consideration of additional training and targeted re-review of study slides. In pilot studies with experienced readers, there was concordance in 85/94 (90.5%) of submitted cases; discrepancies primarily included false positives for hemozoin due to artifact, and false negatives due to low levels of hemozoin. We anticipate that a standardized histopathology QC/QA protocol will be of value to pregnancy malaria research community, and have potential to strengthen local histopathology expertise.

1809

IMPACT OF MALARIA RAPID DIAGNOSTIC TESTS ON CARE OF FEBRILE PATIENTS: CROSS-PROJECT RESULTS FROM THE ACT CONSORTIUM

Baptiste Leurent, Katia Bruxvoort, Eleanor Grieve, Eleanor Hutchinson, Joanna Reynolds, Toby Leslie, ACT Consortium

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

The introduction of malaria rapid diagnostic tests (RDTs) is aimed to increase the proportion of febrile patients tested for malaria, but there are gaps in knowledge of what happens to patients who receive an RDT compared to those who do not. Multi-project data from the ACT Consortium provide an opportunity to examine the impact of RDTs on patient care outcomes under different contexts and settings. Twelve projects from seven countries contributed data, including projects in public health facilities, private retailers, and community health worker settings. Each compared different sets of diagnostic methods, resulting in 30 arms for analysis, which were grouped into four diagnostic categories: presumptive, microscopy, RDT, or enhanced RDT (in which RDTs were provided alongside supportive interventions). Patient care outcomes such as RDT use, antimalarial prescription, antibiotic prescription, referrals, patient satisfaction, and consultation out-of pocket costs were summarised for each arm and compared between categories. Preliminary results indicate a lower prescription of ACTs and a generally higher prescription of antibiotics where RDTs were used, compared to arms where only presumptive diagnosis was available. This difference was more marked in enhanced RDT arms. Referral to higher level care was also more frequent among RDT arms. However, the impact of RDTs was not always consistent and may depend on study setting and design, type of sector, characteristics of patients and providers, and measures of RDT implementation and support. Exploration of these contextual factors is underway, with complete results expected to be available in August 2014. While many studies have reported a positive impact of RDTs on patient outcomes, the effect is complex and likely to vary by setting and context. Further scrutiny is needed to better understand the impact of RDTs on patient care beyond their role as an important diagnostic tool.

1810

SCHISTOSOME POPULATION GENOMICS USING SINGLE ARCHIVED MIRACIDA

Frédéric Chevalier[1], Winka Le Clec'h[1], Philip LoVerde[2], Rafael Ramiro de Assis[3], Guillerme Corrêa-Oliveira[3], Safari Kinunghi[4], Anouk Gouvras[5], Bonnie Webster[5], Joanne P. Webster[6], Aidan Emery[5], David Rollinson[5], Tim Anderson[1]

[1]Texas Biomedical Research Institute, San Antonio, TX, United States, [2]Departments of Biochemistry and Pathology, University of Texas Health Science Center, San Antonio, TX, United States, [3]Centro de Pesquisas René Rachou-Fiocruz, Belo Horizonte, Brazil, [4]National Institute for Medical Research, Mwanza Centre, Mwanza, United Republic of Tanzania, [5]Wolfson Wellcome Biomedical Laboratories, Department of Life Sciences, Natural History Museum, London, United Kingdom, [6]Department of Infectious Disease Epidemiology, School of Public Health, Imperial College Faculty of Medicine, London, United Kingdom

Population genomic analyses of schistosomes have not previously been possible due to the difficulty of sampling adult worms and the high repeat content and the large size (360-400Mb) of their genomes. We have developed a robust, inexpensive approach for capture and sequencing of the ~15Mb *Schistosoma mansoni* exome that can be used for single larval miracidia. The approach uses whole genome amplification of miracidia preserved on FTA cards, solution-based capture of exome sequences using 120bp RNA baits, and Illumina sequencing, and can be extensively multiplexed to reduce costs and simplify sequence library preparation. To demonstrate the utility of these methods we sequenced exomes from 45 single miracidia collected from a Brazilian location in three lanes of an Illumina HiSeq. We captured >99% of the exome sequences targeted,

obtained between 30-80x read depth per miracidia, and robustly called >70,000 SNPs. The method also efficiently captures exomes from the related parasite, S. rodhaini, providing outgroup sequences and opening up the possibility of detailed dissection of interspecific hybridization within schistosome populations. We are currently using these methods to characterize African *S. mansoni* populations from the SCAN collection at the British Natural History Museum. The exome data will be used to characterize SNP variation at candidate vaccine and drug resistance loci, to examine geographic differentiation in allele frequencies, and to identify genome regions under strong directional and balancing selection. We believe that this approach will have multiple uses for schistosome epidemiology, population biology and evolutionary genomics.

1811

POPULATION AND COMPARATIVE GENOMICS OF AFRICAN *SCHISTOSOMA MANSONI*

Thomas Crellen[1], James Cotton[2], Sophia David[2], Caroline Durrant[2], Thomas Huckvale[2], Nancy Holroyd[2], Fiona Allen[3], David Rollinson[3], Joanne Webster[1], Matthew Berriman[2]

[1]Imperial College, London, United Kingdom, [2]Wellcome Trust Sanger Institute, Cambridge, United Kingdom, [3]Natural History Museum, London, United Kingdom

Schistosomiasis is among the most important parasitic diseases, with over 200 million people infected and 300,000 deaths annually across Africa, Asia, South America and the Caribbean caused mainly by three closely related species. Around 90% of cases are in sub-Saharan Africa, where *Schistosoma mansoni* is one of the two most clinically important species, and the principal cause of intestinal schistosomiasis. A draft reference genome is available for *S. mansoni* and is being actively curated and improved, based on an isolate from Puerto Rico that has been maintained in research labs for many years. Here, we present genome sequence data and assemblies from seven adult male *S. mansoni* that were recently collected from the field with minimal lab passage, including six diverse African isolates - the first genomic data from the region of greatest public health interest. We confirm that the *S. mansoni* reference sequence is a suitable substrate for genomic analysis of African populations. We use this genomic diversity data to investigate signatures of natural selection on the *S. mansoni* genome, and apply two coalescent-based models to infer the population history of *S. mansoni* on two continents. Our results show that the New World strains have smaller past effective population sizes (Ne) than African strains, suggesting the possible occurrence of a past population bottleneck. We estimate the divergence time between the African and New World populations, finding support for the hypothesis that *S. mansoni* colonised the New World via the 16-19th century West African slave trade. In the light of this potential population bottleneck, we investigate systematic differences between South American populations and African populations in both genome structure (copy number variants) and single nucleotide polymorphisms (SNPs).

1812

USING MICROSATELLITE MARKERS TO DETERMINE *SCHISTOSOMA MANSONI* GENETIC DIVERSITY UNDER CONTRASTING CHEMOTHERAPY CONTROL STRATEGIES IN LAKE VICTORIA, TANZANIA

Anouk N. Gouvras[1], Bonnie L. Webster[2], Fiona Allan[1], Muriel Rabone[1], Safari Kinunghi[3], Aidan Emery[1], Joanne P. Webster[2], David Rollinson[1]

[1]Natural History Museum, London, United Kingdom, [2]Imperial College, School of Public Health, London, United Kingdom, [3]National Institute for Medical Research, Mwanza, United Republic of Tanzania

National schistosomiasis control programs rely on mass drug administration using the drug praziquantel (PZQ). The widespread use of a single drug raises concerns of drug tolerance spreading in the parasite population. In the absence of specific markers for PZQ tolerance, neutral genetic markers such as microsatellite loci, and analysis of population genetics can be used to monitor changes in parasite populations and the effect of PZQ-reliant schistosomiasis control on the parasite's adaptation and evolution. In the Lake Victoria region of Tanzania (Mwanza), as part of a larger treatment study on intestinal schistosomiasis caused by *Schistosoma mansoni*, we are collecting parasite genetic material from school children from 16 villages with contrasting PZQ treatment pressures: annual Community-Wide Treatment (CWT-highest treatment pressure) and biennial School-Based Treatment (SBT-lowest treatment pressure). The infection prevalence at the start of the study was over 25%. In each village larval miracidia samples were collected prior to treatment from infected school children (n=30). For baseline (2012) 18,649 *S. mansoni* miracidia were collected from 263 children in 16 villages. In Year 2 (2013), 4,724 *S. mansoni* miracidia were collected from 95 children in the 8 annual CWT villages. Future follow-up collections are planned for May 2014 (16 villages). Samples were collected on to FTA cards and stored in SCAN (http://scan.myspecies.info/). Samples are being analysed using a new set of multiplex panels developed from 20 previously published microsatellites for *S. mansoni*. Comparison of genetic diversity indices such as number of alleles per locus, allelic richness, observed and expected heterozygosity will be made between baseline and post treatment samples and between annual CWT and biennial SBT control intervention strategies. Initial analysis of baseline-collected material shows little difference in the genetic diversity indices between the two different treatment arms. Year 2 miracidia samples are currently being analysed to determine if there has been a change in genetic diversity compared to baseline in the 8 CWT villages. This study utilizes field-collected material and microsatellite multiplex panels to determine the differential impact of annual CWT vs. biennial SBT chemotherapy strategies on parasite clearance and population genetic outcomes.

1813

REPEATED TREATMENTS ARE REQUIRED TO AFFECT SCHISTOSOME POPULATION STRUCTURE

Lucio M. Barbosa[1], Luciano K. Silva[1], Eliana A. Reis[1], Theomira M. Carmo[1], Claudio R. dos Santos[1], Jackson M. Costa[1], Peace T. Aminu[2], Thassila N. Pitanga[1], Walter A. Blank[2], Mitermayer G. Reis[1], Ronald E. Blanton[2]

[1]Oswaldo Cruz Foundation, Salvador, Brazil, [2]Case Western Reserve University, Cleveland, OH, United States

Repeated rounds of praziquantel treatment are able to reduce prevalence and morbidity of schistosomiasis, but parasite populations recover within a few years. To understand the dynamics of this recovery, two rural Brazilian communities were surveyed and treated in 3 different years: 2009, 2012 and 2013. On average, 96% of the residents participated in each year, and those who were positive on at least one of 3 stools were treated. *Schistosoma mansoni* eggs were collected from stool and genotyped using 11 microsatellite markers. Parasite differentiation was evaluated at the level of infrapopulations and component populations. Component populations were defined by host characteristics, village of residence or year of study. During this time, human population demographics changed little. New arrivals were 16% and 5% of the populations in 2012 and 2013, respectively. In both years ~12% moved elsewhere. After 2 rounds of treatment, prevalence decreased by 64% and intensity by 57%. Children 15-20 years old showed the greatest decline, while adults between 51-60 showed the least. Reinfection was 34% in 2012 and 18% in 2013, while incidence was 22% and 15%, respectively. The decreasing rates suggest that these treatments have an effect on transmission. Individual infrapopulations were moderately differentiated (D=0.055-0.077) on reinfection, indicating the pre-treatment multilocus genotypes were not fully reacquired. Differentiation between the 2 villages decreased from 0.046 to 0.031, consistent with an increase in gene flow between them. Parasites from new immigrants were little differentiated from natives (D = 0.012). Between consecutive years, there was little differentiation (D = 0.008), but comparing 2009 to 2013, differentiation increased notably (D = 0.014). Population structure began to change only after 2 rounds of treatment when total parasite burden decreased by >10 fold. This seems

to be the tipping point for producing a genetic bottleneck or reducing effective population size. Intensive therapy is required to significantly impact the parasite's genetic potential.

1814

EPIGENETIC CONTROL OF ENDOGENOUS AND EXOGENOUS (RETRO)TRANSPOSABLE ELEMENTS IN *SCHISTOSOMA MANSONI*

Hong-Bin Yan, Paul J. Brindley, **Gabriel Rinaldi**

George Washington University, Washington, DC, United States

The epigenetic landscape includes modifications on the chromatin template that establish and propagate patterns of gene expression and silencing, not based on differences in the DNA sequence. Four major epigenetic phenomena are involved: 1) DNA methylation; 2) histone modifications; 3) nuclear gene repositioning; and 4) regulatory non-coding RNAs. Whereas early reports suggested absence of DNA methylation of the schistosome genome, it has now been shown that cytosine methylation regulates schistosome oviposition and embryo development. In addition, post-translational core histone modifications in epigenetic control of transcription of schistosome genes have been described, as have a set of non-coding RNAs -- integral components of the epigenetic machinery, including epi-miRNAs, i.e. miRNAs that regulate expression of enzymes involved in chromatin remodeling and DNA methylation. To evaluate the effect of the DNA methyltransferase inhibitor 5′-azacytidine (5′-AzaC) on the expression of both long terminal repeat (LTR) and non-LTR retrotransposons, schistosomules of *Schistosoma mansoni* were cultured in 100 µM and 500 µM of 5′-AzaC. The parasites were harvested 2 or 7 days after treatment, RNA was isolated and the expression of the *Boudicca* (LTR-retrotransposon) and *SR2* (non-LTR-retrotransposon) multi-copy endogenous mobile genetic elements of schistosomes analyzed by qRT-PCR. The expression level of these retrotransposons was upregulated 10 to 20 times in the presence of 5′-AzaC. In addition, expression of reporter transgenes increased in schistosomules that had been transformed with virions of pseudotyped murine leukemia virus following culture in media supplemented with 5′-AzaC. These findings demonstrated that endogenous mobile elements, which comprise ~45% of the genome of this schistosome are controlled by epigenetic marks. In addition, they indicated a central influence of methylation status on transgene activity, suggesting one avenue forward for enhancing transgenesis of this tropical neglected tropical disease pathogen.

1815

SCHISTOSOMA MANSONI: ROLE OF BIOGENIC AMINES IN NEURONAL CONTROL OF MOTOR FUNCTION

Nelly El-Sakkary, Paula Ribeiro

McGill University, Ste. Anne-de-Bellevue, QC, Canada

Schistosoma mansoni is the main causative agent of schistosomiasis, a disease which infects over 200 million people worldwide. Treatment of the disease is primarily with praziquantel. With the lack of an available alternative, and the widespread use of the drug, there is a fear of the development of resistance. Biogenic amines (BAs) are the largest family of classical neurotransmitters in the schistosome nervous system. They are typically involved in motor control and are important to host infection and worm survival. The goal of this study is to determine the role of BA neurotransmitters in schistosomes, focusing on tyrosine derivatives, which include phenolamines and catecholamines. Using confocal immunolocalization, receptors responsive to the catecholamine, dopamine (DA), SmGPR3 and SmD2, were shown to localize to the main nerve cords of the central nervous system (CNS), and the peripheral nervous system (PNS), respectively. Both receptors localized to neurons innervating worm musculature, indicating a possible role in motility for the receptors. Octopamine (OA), a phenolamine, was also immunolabeled in the adult parasite, and showed widespread labeling in the main neurons of the worm CNS, the first indication that OA is present in schistosomes. In other studies we tested the role of several BAs on schistosomes motility. Treatment with both OA and DA caused marked changes in worm motility as compared to the control. Next, we performed RNAi targeting proteins predicted to be involved in DA and OA signaling in larvae and adult schistosomes, and effects of downregulation were assessed. Several of the RNAi-targeted animals showed strong changes in frequency of body movements and in worm morphology as compared to the control. Together these studies highlight the importance of tyrosine derived BAs in the control of motor activity in schistosomes.

1816

MICRORNAS IN THE EXCYSTATION OF *FASCIOLA HEPATICA* METACERCARIAE

Jose F. Tort[1], Santiago Fontenla[1], Nicolas Delloca[1], Pablo Smircich[1], Maria del Mar Siles-Lucas[2]

[1]Universidad de la Republica, Montevideo, Uruguay, [2]Instituto de Recursos Naturales y Agrobiología, Salamanca, Spain

Host invasion by the trematode parasite *Fasciola hepatica* is initiated by the activation of the metacercariae usually in the host stomach. The released juvenile forms actively transverse the gut wall towards the abdominal cavity and follow their journey to the biliary ducts of the liver. The activation is a rapid switch finely tuned by signals from the environment, that can be easily reproduced *in vitro*. miRNAs have emerged as relevant modulators of gene expression at the post-transcriptional level (either by tranlation blocking or mRNA degradation), playing essential roles in development. We aimed to study the miRNA expressed in the liver fluke metacercarial activation, and for that purpose we purified and sequenced the small RNA populations expressed at this developmental transition. After filtering the reads with homology to mRNA, repetitive sequences and other non coding RNAs, we ended up with several thousand reads that were compared to miRBase, Rfam and all the miRNA previoulsy identified in other flatworms. Within the known miRNAs found, those common to all metazoans and protostomes were the most abundant. Some miRNA so far only detected in other flatworms were also found, highlighting the existance of flatworm specific miRNA families. Furthermore within sequences with no homology, novel *F. hepatica*-specific miRNAs were predicted. We observed subtle differences between dormant and activated metacercariae and further differences to newly excysted juveniles. While sequence conservation in mature miRNA is high across the metazoan tree, we observed that in general flatworm miRNA are more divergent than in other lineages, with strict conservation restricted to seed region. Whether this variability leads to changes in the regulated target genes associated to the parasitic way of life deserves further investigation.

1817

IDENTIFICATION OF SYMBIONTS IN THE REPRODUCTIVE TRACT MICROBIOME OF NATURAL *ANOPHELES GAMBIAE* POPULATIONS AND IMPLICATIONS FOR NOVEL VECTOR CONTROL METHODS

Francesco Baldini[1], Nicola Segata[2], Julien Pompon[3], Perrine Marcenac[4], W. Robert Shaw[5], Roch K. Dabiré[6], Abdoulaye Diabaté[6], Elena A. Levashina[7], Flaminia Catteruccia[4]

[1]University of Glasgow, Glasgow, United Kingdom, [2]University of Trento, Trento, Italy, [3]University of Strasbourg, Strasbourg, France, [4]Harvard School of Public Health, Boston, MA, United States, [5]Imperial College London, London, United Kingdom, [6]Institut de Recherche en Sciences de la Santé/ Centre Muraz, Bobo-Dioulasso, Burkina Faso, [7]Max-Planck Institute for Infection Biology, Berlin, Germany

Identification of symbionts in the reproductive tract microbiome of natural *Anopheles gambiae* populations and implications for novel vector control methods Recent findings show that mosquito-microbiota interactions are crucial determinants of mosquito fitness and vectorial capacity opening novel and promising areas of research in the utilization of symbiotic bacteria for the control of vector-borne diseases. Furthermore, recent

works propose the use of paratransgenesis as a novel vector control tool, which exploits genetically engineered symbionts to deliver anti-parasite molecules in the vector. In order to identify potential determinants of mosquito biology as well as putative candidates for paratransgenesis, we have characterized the reproductive microbiome of two major African malaria vectors *Anopheles gambiae* and *An. coluzzii* from field population in Burkina Faso. Specifically, we performed high throughput sequencing of the bacterial 16S gene in the male and female reproductive tracts of mosquitoes. We identified two bacteria genera that are present in all the analysed specimens, representing the core taxa of mosquito reproductive organs with possible symbiotic interactions with the vector. Nevertheless, although a general core microbiome was identified, we observed a general high diversity among different specimens that might indicate that the reproductive microbiome is highly dynamic and might be influenced by external factors. Indeed, we identified some taxa whose abundance was significantly associated with the environment where the mosquitoes lived. Finally, we identified intracellular bacteria that were previously believed not to colonize natural populations of *Anopheles*. Remarkably, these bacteria are capable of spreading into insect populations and negatively impact mosquito vectorial capacity by reducing their lifespan and boosting the immune response against parasites. To our knowledge this was the first identification of these intracellular bacteria in malaria mosquitoes, which opens new promising opportunities to exploit these organisms as vector control agents against malaria vectors. These results started to elucidate the composition of malaria mosquito reproductive tract microbiome offering novel opportunities to exploit symbiotic bacteria in the fight against malaria.

1818

PERIOSTIAL HEMOCYTE AGGREGATION IN *ANOPHELES GAMBIAE* OCCURS FOLLOWING DIVERSE IMMUNE STIMULI AND IS ACCOMPANIED BY CHANGES IN MOSQUITO HEART PHYSIOLOGY

Julian F. Hillyer, Leah T. Sigle, Tania Y. Estevez-Lao

Vanderbilt University, Nashville, TN, United States

Malaria parasites and other mosquito-borne pathogens must traverse the insect hemocoel prior to being transmitted. This process is affected by hemolymph circulation, as flow influences both pathogen movement and the movement of mosquito-produced immune factors. Using the African malaria mosquito, *Anopheles gambiae*, as our study system, we recently identified a novel immune tissue, called periostial hemocytes, that exemplifies the co-adaptation of the insect immune and circulatory systems. Specifically, in response to infection, circulating hemocytes (immune cells) migrate to the valves of the mosquito heart, where they sequester and kill pathogens. We have previously reported the aggregation of hemocytes on the surface of the heart following *Plasmodium* and *Escherichia coli* infection, however, little is known about the breadth of this immune response or about how heart physiology changes following infection. In the present study we tested whether periostial hemocyte aggregation occurs following diverse immune stimuli, whether this response is uniform across the length of the heart, and whether infection affects mosquito heart physiology. We found that periostial hemocyte aggregation occurs following all types of infections tested, confirming the fundamental role of this immune response. Moreover, periostial hemocyte aggregation is not uniform along the length of the heart, as larger hemocyte aggregates consistently form in abdominal segments 4, 5, and 6. Finally, periostial hemocyte aggregation is accompanied by a decrease in heart contraction rates. In summary, these data further describe a recently discovered immune tissue in mosquitoes, and demonstrate how the immune and circulatory systems have co-adapted to fight infection.

1819

BREAKING THE LAW OF EFFECTIVE TEMPERATURE: ECOLOGICAL CONTEXT MATTERS FOR DEVELOPMENT RATE VARIATION MOSQUITO VECTORS

Jannelle Couret[1], Ellen Dotson[2], Mark Q. Benedict[3]

[1]Emory University, Atlanta, GA, United States, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]University of Perugia, Perugia, Italy

The resurgence and spread of arboviruses in recent years underscores the continued need to understand the biology of its mosquito vectors including, *Aedes aegypti* and *Culex pipiens* complex. Despite advances in the development of vaccines, control of the mosquito vector populations remains the most effective control measure. In order to understand how the environment impacts disease transmission, a thorough understanding of the impact of environmental variables on mosquito biology is needed. Historically, the emphasis has been on temperature and the linear association with insect development rate. This association is widely observed across ectotherms, and is often referred to as the law of effective temperature. We tested the hypothesis that the law of effective temperature is contingent on the ecological context of the larval environment in mosquito development. Through a combination of statistical modeling of published rearing experimental research and a larval rearing experiment under gradients of conditions in environmental chambers, we find that intraspecific density and dietary resources mediate the importance of temperature in explaining variation of mosquito development rate. Our results support the hypothesis of environmentally contingent impacts of temperature on mosquito development. These findings have broad implications for the modeling of mosquito population dynamics, climate change and vectorborne disease transmission, and our understanding of nature of mosquito life history evolution.

1820

PARASITE CO-INFECTION AND STRAIN DIFFERENCES AS DRIVERS OF PATHOGENIC VARIATION IN THE CHAGAS DISEASE PARASITE *TRYPANOSOMA CRUZI* WHEN INFECTING ITS INSECT VECTOR, *RHODNIUS PROLIXUS*

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

[1]Princeton University, Princeton, NJ, United States, [2]University of Antioquia, Medellín, Colombia

Avoiding the over-exploitation of resources in a food patch while still obtaining all the resources needed to live and reproduce is a common challenge among all creatures. For many parasites that live inside another organism this is especially tricky because they do not have the option of moving to a new patch should they be too pathogenic and overexploit their current one. Classical theory suggests that the avoidance of host death should favor intermediate levels of parasite pathogenicity (i.e., negative effects) to the host, however that this is not the case: parasites actually exhibit a wide range of pathogenic effects on their hosts. We investigated parasite co-infection and strain differences as drivers of pathogenic variation in the Chagas disease parasite *Trypanosoma cruzi* and its sister species, *Trypanosoma rangeli* when infecting their insect vector, *Rhodnius prolixus.* Using insect survival, reproduction, and parasite load (qPCR amplification of parasite DNA extracted from each insect) as proxies for parasite pathogenicity, we found that *T. cruzi-T. rangeli* co-infection significantly reduces the survival of *R. prolixus* up to 30 days post-infection, but increases reproduction. We also found that *T. cruzi* pathogenicity in *R. prolixus* is highly variable, with *R. prolixus* death at 90 days ranged from 5-80% depending on *T. cruzi* strain, and at times was far more pathogenic than *T. rangeli*, a parasite believed to be highly pathogenic to triatomines. Furthermore, insects with higher parasite loads tended to have higher fecundity, presenting evidence for terminal investment in infected bugs.

Our results suggest that the pathogenic variation often found in *T. cruzi* infection of vertebrate hosts extends to its invertebrate hosts as well, with strain variability and co-infection with *T. rangeli* as two of the main drivers.

1821

THE EFFECTS OF FORCED-EGG RETENTION ON THE BLOOD-FEEDING BEHAVIOR AND REPRODUCTIVE POTENTIAL OF *CULEX PIPIENS* (DIPTERA: CULICIDAE)

Brian J. Johnson[1], Dina M. Fonseca[2]

[1]Graduate Program in Ecology and Evolution, Rutgers University, New Brunswick, NJ, United States, [2]Center for Vector Biology, Department of Entomology, Rutgers University, New Brunswick, NJ, United States

High rates of West Nile virus (WNV) transmission to humans are associated with exceptionally hot and dry summers. This is paradoxical since the eggs of *Culex* vectors of WNV depend on the persistence of containers with water, which decline during droughts. We examined the effects of forced-egg retention on the reproductive success of female *Culex pipiens* as well as behavioral responses, such as likelihood of secondary blood meals. As controls we examined the effects of female age and delayed mating. We found that early mating is essential to achieve reproductive success and, consistent with an "all-or-none" ovipositing strategy, *Cx. pipiens* females are able to retain considerable reproductive potential while searching for oviposition sites. Specifically, although forced-egg retention resulted in significant decreases in fitness, the decline was moderate for 5 weeks and most can be accounted for by increases in female age. Consequently, no females took blood more than once per gonotrophic cycle, which eliminates the possibility that heightened vectorial capacity due to multiple blood-feedings increases WNV transmission during periods of drought. Instead, our findings suggest that during droughts populations of *Cx. pipiens* have time to locate the remaining water holes, which are associated with human populations and WNV-competent bird species.

1822

BEHAVIORAL CHANGE IN *ANOPHELES FUNESTUS*: AN OBSTACLE TO MALARIA ELIMINATION

Sougoufara Seynabou[1], Diedhiou Mocote Seynabou[1], Doucoure Souleymane[1], Trape Jean François[1], Sokhna Cheikh[1], Sembene Mbacke[2], Harry Myriam[3], Ndiath Mamadou Ousmane[4]

[1]IRD, Dakar, Senegal, [2]University, Dakar, Senegal, [3]LEGS-CNRS, Paris, France, [4]Institute Pasteur, Bangui, Central African Republic

In the long road that leads towards malaria elimination, vector control was undoubtedly an essential component of success. Two major strategies have marked the vector control: the use of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). However, the effectiveness of these tools is being challenged by the emergence of insecticide resistance and behavioural resistance which thwarting the goal of decreasing malaria transmission. In this study, we focus on behavioural changes of malaria vectors that can hamper the efficacy of vector control interventions in Dielmo, a Senegalese rural village where a longitudinal study of malaria has been conducted. In this village, universal coverage with LLINs was done in July 2008, and in July 2011 all these LLINs were renewed. Adult mosquitoes were collected by human landing catches (HLC) from July 2011 to April 2013 and hourly from 19:00 and 07:00. Collecting mosquitoes was also done by pyrethrum spray catch (PSC) during this period. From January to April 2013, mosquito catches were continued until 11:00 and the entomological different parameters were investigated. This study shows that *Anopheles funestus* which have disappeared after first introduction of LLINs (July 2008) comes back in malaria transmission in Dielmo. *An. funestus* remains anthropophilic and endophilic but adopt a behavioural change in biting activity after introduction of LLINs. The human biting rate of mosquitoes collected from 07:00 to 11:00 was eight times higher than the one from 19:00 to 07:00. So the alarming phenomenon is the positive mosquitoes found in the day capture (mean CSP rate of 1.28%) while since distribution of LLINs in this village, no *An. funestus* has been found positive to CSP. These disturbing observations show the capacity of *Anopheles* to adapt and circumvent strategies aimed at reducing malaria transmission. In an arms race between malaria control programs and the vector populations, the behaviour change in *Anopheles* threatens to thwart the goal of decreasing malaria transmission.

1823

INFECTION OF LABORATORY-COLONIZED *ANOPHELES DARLINGI* MOSQUITOES BY *PLASMODIUM VIVAX* AND TEMPORAL CHANGE IN GENETIC VARIATION IN *AN. DARLINGI*

Marta Moreno Leirana[1], Carlos Tong[2], William Lainhart[3], Mitchel Guzman[2], Raul Chuquiyauri[2], Alejandro Llanos-Cuentas[4], Hugo Rodriguez[5], Dionicia Gamboa[4], Jan E. Conn[6], Joseph M. Vinetz[1], Joseph M. Vinetz[2], Joseph M. Vinetz[4]

[1]University of California San Diego, La Jolla, CA, United States, [2]Laboratorio ICEMR-Amazonia, Laboratorios de Investigacion y Desarrollo, Facultad de Ciencias y Filosofia, Universidad Peruana Cayetano Heredia, Lima, Peru, [3]Department of Biomedical Sciences, School of Public Health, State University of New York-Albany, Albany, NY, United States, [4]Instituto de Medicina Tropical "Alexander von Humboldt", Universidad Peruana Cayetano Heredia, Lima, Peru, [5]Loreto Regional Directorate of Health, Iquitos, Peru, [6]Wadsworth Center, New York State Department of Health, Albany, NY, United States

Anopheles darlingi Root is the most important malaria vector in the Amazonia region of South America. However, continuous propagation of *An. darlingi* in the laboratory has been elusive, limiting entomological, genetic/genomic, and vector–pathogen interaction studies of this mosquito species. We report the establishment and maintenance of an *An. darlingi* colony (since July 2013) derived from wild-caught mosquitoes obtained in the northeastern Peruvian Amazon region of Iquitos in Loreto Department. We demonstrate that the numbers of eggs, larvae, pupae, and adults continue to rise at least to the F9 generation. In addition, comparison of feeding *Plasmodium vivax* by artificial membrane feeding of F4-F9 to F1 generation mosquitoes showed the comparable presence of oocysts and sporozoites, with numbers that corresponded to blood-stage asexual parasitemia and gametocytemia, confirming *P. vivax* susceptibility in the colonized mosquitoes. Additionally, analyses of fourteen microsatellites markers were performed on a subsample of *An. darlingi* offspring to detect genetic variation and expected reduction in heterogeneity through generations. These results provide new avenues for research on *An. darlingi* biology and study of mosquito–*Plasmodium* interactions and malaria transmission in the Neotropics, including new genomic analysis and assessment of transmission biology of malaria parasites.

1824

RATIONAL DESIGN OF OXAMNIQUINE DERIVATIVES THAT KILL SCHISTOSOMES

Stacey Stahl[1], Alexander B. Taylor[1], Xiaohang Cao[1], Stanton McHardy[2], P. John Hart[1], Timothy J. Anderson[3], **Philip T. LoVerde**[1]

[1]University of Texas Health Science Center, San Antonio, TX, United States, [2]University of Texas at San Antonio, San Antonio, TX, United States, [3]Texas Biomedical Research Institute, San Antonio, TX, United States

Schistosomiasis, a major cause of morbidity, infects >200 million people worldwide. Schistosomiasis control is based on a monotherapy consisting of repeated doses of praziquantel (PZQ). Drug resistance is a concern, especially as it is expected to increase in treatment coverage in sub-Sahara Africa (250 million doses per year for each of the next 5 years). New anti-schistosomal drugs are needed to reduce reliance on a single drug. A new drug could be used in combination with PZQ to minimize the probability of resistance arising to either drug. The goal of this research is to modify an existing anti-schistosomal drug oxamniquine (OXA) to make it more efficacious. We recently identified the gene encoding the Sm-sulfotransferase (SmSULT) responsible for drug activation and determined

the structure of the SmSULT•cofactor•OXA ternary complex at 1.75 Å resolution. These analyses provide detailed information of the mechanism of action of OXA against Sm, while structural analyses of drug•protein interactions direct redesign of OXA. We designed and synthesized 12 OXA derivatives based on four key design aspects; 1) the structural requirements of OXA and its derivatives based on available space in the substrate binding cavity in SmSULT and the key residue interactions from crystallographic studies, 2) the required ortho-electron withdrawing moiety necessary for the sulfonation process, 3) the design of analogs that fall within favorable "drug-like" physical chemical property ranges and 4) the development of efficient and convergent syntheses, allowing for the greatest amount of structural diversity and chemical space to establish structure-activity relationships (SAR). *In vitro* worm killing assays indicated that three of these analogs were as good as or better than OXA itself. These new compounds with antischistosomal activity have been soaked into the SmSULT•PAP crystals and their mode of binding elucidated. This information will be used to synthesize the next generation of OXA derivatives.

1825

ROLES OF ATP BINDING CASSETTE (ABC) MULTIDRUG TRANSPORTERS IN SCHISTOSOME PHYSIOLOGY, DRUG SUSCEPTIBILITY AND PARASITE-HOST INTERACTIONS

Ravi S. Kasinathan, Yue Ning, **Robert M. Greenberg**

University of Pennsylvania, Philadelphia, PA, United States

Although its value in the treatment and control of schistosomiasis is well established, praziquantel (PZQ) has significant limitations. Most notably, it is largely ineffective against immature schistosomes. It is also essentially the only drug available for a disease afflicting hundreds of millions. New therapeutics or adjuncts to enhance PZQ activity and overcome possible drug resistance are urgently needed. We hypothesize that ATP binding cassette (ABC) multidrug transporters offer attractive candidate targets for new or repurposed drugs that either act as anthelmintics on their own, or that enhance parasite susceptibility to existing anthelmintics. ABC transporters such as P-glycoprotein (Pgp) mediate efflux of metabolic toxins, xenobiotics, and signaling molecules, and are associated with drug resistance in many organisms, including parasitic helminths. They exhibit broad substrate specificity and are inhibited by several drugs currently in clinical use. ABC transporters are also implicated in a variety of normal physiological activities such as excretion, maintenance of permeability barrier function, and modulation of immune responses. They transport many potent signaling molecules with high affinity, including several with immunomodulatory activity. Schistosomes exposed to PZQ increase expression of ABC transporters such as Pgp (SMDR2) and multidrug resistance associated protein (SmMRP1), and worms with reduced PZQ sensitivity show higher basal expression of these transporters. PZQ is also both an inhibitor and likely substrate of schistosome Pgp. Disruption of transporter expression (by RNAi) or function (by inhibition) enhances the activity of PZQ against adult parasites, and renders PZQ-refractory juvenile worms susceptible to the drug. Schistosome ABC transporters also appear to be important for normal schistosome egg production. We are currently exploiting molecular and pharmacological tools to understand the mechanism by which schistosome ABC transporters alter PZQ susceptibility and to assess the role of these transporters in the parasite's modulation of host immune responses. These experiments could lend important insights into schistosome physiology and possibly provide targets for novel antischistosomals.

1826

SHORT-TERM ANTIBIOTIC TREATMENT INTERRUPTS THE EXCHANGE OF POLYMORPHIC VESICLES BETWEEN *WOLBACHIA* AND THEIR FILARIAL HOST

Kerstin Fischer, Peter U. Fischer

Washington University School of Medicine, St. Louis, MO, United States

Wolbachia endosymbionts are crucial for growth, reproduction, and survival of many medically important filarial parasites. *Wolbachia* have a tightly regulated lifecycle within filarial nematodes. While *Wolbachia* density is low in microfilariae and vector stage larvae, much higher numbers of the endobacteria are observed in developing larvae and young adult worms recovered from mammalian hosts. We have recently reported that *Wolbachia* release polymorphic outer membrane vesicles (OMV) that may be essential for their mutualistic relationship with filarial worms. OMV may transport bacterial products that are required for parasite growth and development. Tetracyclines (TET, antibiotics that inhibit bacterial protein synthesis) clear *Wolbachia* from filarial worms over a period of weeks. This treatment first sterilizes the parasites and eventually kills them. The present study was performed to elucidate the early effects of TET treatment on the morphology of *Wolbachia* and filarial worms. Gerbils with i.p. *Brugia malayi* infections were treated with TET on days 19 and 20 post-infection (i.p. injection, 5 mg/kg). Immature female worms recovered on day 21 were studied by transmission electron microscopy using high pressure freezing/freeze substitution fixation. OMV were largely absent near *Wolbachia* in TET-treated worms, while about half of the *Wolbachia* in untreated control worms were associated with OMV. *Wolbachia* in treated worms were often surrounded by membranes that appeared to come from the endoplasmic reticulum. Lateral chords in treated worms were heavily vacuolated with increased glycogen granules compared to untreated worms. Thus TET treatment appears to block OMV production by *Wolbachia* and promote encapsulation of the bacteria by internal host cell membranes.

1827

POPULATION GENOMICS OF *WUCHERERIA BANCROFTI* ELIMINATION FROM PAPUA NEW GUINEA

Scott T. Small[1], David Serre[2], James F. Hester[2], Lisa J. Reimer[3], Daniel J. Tisch[1], Peter A. Zimmerman[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]Cleveland Clinic, Cleveland, OH, United States, [3]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Lymphatic filariasis (LF) is a major threat to human health in the tropics, leading to the disfiguring and debilitating conditions hydroceles and elephantiasis. There are approximately 1.2 billion people living in LF endemic countries where 120 million people currently suffer from the disease. The parasites that cause LF are separated into two genera, *Brugia* and *Wuchereria*, with *W. bancrofti* (Wb) responsible for ~90% of LF cases. Until recently, Wb has been neglected in genomic studies due to complications in sample collection and preparation, such as lack of adult stage samples. Here, we report 13 new Wb genomes from the Dreikikir region of Papua New Guinea (PNG). We utilized multiple displacement amplification to amplify and sequence 13 juvenile stage (L3) Wb worms from four patient infections. We report the discovery of 60,000 novel single nucleotide polymorphism (SNPs) and 200 polymorphic microsatellite loci from the genomes. Within patient infections we find that genetic diversity is high, yet concentrated in specific regions of the genome, with large tracts of intervening homozygous sequence. We also identify candidate regions that harbor genes with extended haplotypes and shifted frequency spectrums, signals of either ongoing or recent positive selection. We discuss our results in the context of the recent mass drug administration, and identify SNP loci ideal for future monitoring of elimination success.

1828

NOVEL TEGUMENT EXPRESSED KUNITZ TYPE PROTEASE INHIBITOR FROM *SCHISTOSOMA MANSONI*

Shiwanthi L. Ranasinghe, Katja Fischer, Geoffrey Gobert, Donald P. McManus

QIMR Berghofer Medical Research Institute, Herston, Australia

Schistosomiasis is one of the most prevalent and serious parasitic diseases of tropical and subtropical regions with 779 million people being at risk and 207 million infected. Kunitz type proteins belong to the I2 family of protease inhibitors and are involved in diverse biological processes in invertebrates. One secretory type sequence (SmKI) having similarity to Kunitz type protease inhibitors was identified from recent mining of the genome of *Schistosoma mansoni*. Recombinant SmKI was expressed in *E. coli,* purified and an antiserum against rSmKI was produced in mice and subsequent immunolocalization and western blotting carried out. Gene expression levels were determined within key lifecycle stages of *S. mansoni* using real-time PCR. Serine protease inhibitory assays were also used to determine the inhibitory effect of the rSmKI on trypsin, chymotrypsin, neutrophil elastase (NE), pancreatic elastase and Cathepsin G. Real time PCR indicated SmKI is highly expressed in adult worms which reside in the mesenteric venules of the definitive host. Immunolocalization showed the Kunitz protein is present in the tubercles of the male tegument and along the tegument of the female worm. Notably, western blots showed the level of SmKI was higher in the excretory secretory products of adult worm pairs than in soluble worm antigens. Further, rSmKI inhibited trypsin, chymotrypsin and NE, with the highest inhibitory activity recorded against trypsin. Initial screens indicated that rSmKI interfere with both intrinsic and extrinsic blood coagulation pathways as well, indicating another important function. Thus, the SmKI protein may play an important role in schistosome survival in blood by inhibiting NE as well as playing a key role in evading host immune responses. As SmKI is secreted and exposed to the host immune system, we consider that rSmKI may be a useful candidate as novel vaccine target to control schistosomiasis. Assays are underway to further understand the function of SmKI and vaccine/challenge experiments will be undertaken to evaluate its protective efficacy.

1829

PLASMODIUM VIVAX LIVER STAGE DEVELOPMENT AND HYPNOZOITE FORMATION IN THE FRG HUHEP MOUSE MODEL

Sebastian A. Mikolajczak[1], Niwat Kangwanrangsan[2], Ashley M. Vaughan[1], Wanlapa Roobsoong[2], Rachaneeporn Jenwithisuk[2], Narathatai Yimamnuaychok[2], Matt Fishbauger[1], Viswanathan Lakshmanan[1], Alexis Kaushansky[1], Scott E. Lindner[3], John H. Adams[4], Jetsumon Sattabongkot[2], Stefan H. Kappe[1]

[1]Seattle BioMed, Seattle, WA, United States, [2]Mahidol University, Bangkok, Thailand, [3]Pennsylvania State University, University Park, PA, United States, [4]University of South Florida, Tampa, FL, United States

The ability of *Plasmodium vivax* to form dormant liver stages (hypnozoites) that can be activated weeks or months after initial infection to cause relapse of malaria is of crucial importance for the unprecedented epidemiological success of the parasite. Yet, little progress has been made to understand the unique biology of hypnozoite formation and activation. Due to the parasite's strong preference for nonhuman primate and human tissue, the availability of models to study *P. vivax* liver stages is extremely limited. Here we report that the FRG KO mouse model transplanted with human primary hepatocytes (huHep) efficiently supports the development of *P. vivax* liver stages as well as the formation of hypnozoites for Thai isolates of *P. vivax*. Using a series of *P. vivax* specific polyclonal and monoclonal antibodies, we were able to evaluate the liver stage progression and maturation in the infected liver. The ability of the exo-erythrocytic merozoites to establish blood stage infection upon transfusion with human reticulocytes is being currently evaluated. Furthermore, *P. vivax* infections in the FRG huHep mice carried beyond the time of the liver stage maturation showed that persistence and activation of *P. vivax* hypnozoites can be further investigated in the model to determine the biological basis for liver stage dormancy. Successful evaluation of the antimalarial drugs with known activities on *P. vivax* liver stage infection (Primaquine and Atovaquone) confirmed that the FRG huHep/*P. vivax* infection model could be used as an efficient platform for testing new antimalarial drugs *in vivo* in quest to accelerate the development of interventions for the radical cure of *P. vivax* infection.

1830

CHARACTERIZATION OF COENZYME A BIOSYNTHESIS PATHWAY REVEAL ESSENTIAL DISTINCTIVE FUNCTIONS DURING *PLASMODIUM* DEVELOPMENT IN BLOOD AND MOSQUITO

Robert J. Hart, Catherine Nation, Panpim Thongsripong, **Ahmed S. I. Aly**

Tulane University, New Orleans, LA, United States

Coenzyme A (CoA) is an essential universal cofactor and carrier of acyl groups for all prokaryotic and eukaryotic cells. In nearly all non-photosynthetic cells, CoA biosynthesis depends on the uptake and phosphorylation of vitamin B5 (pantothenic acid or pantothenate). Earlier Studies showed the importance of pantothenate acquisition and phosphorylation for *Plasmodium* survival within erythrocytes. Recently, pantothenate plasma membrane transporter (PAT) was functionally characterized in *Plasmodium falciparum*. PAT was shown to be refractory to deletion and was localized to the parasite plasma membrane. However, very little is known about the *in vivo* cellular functions of CoA biosynthesis pathway in malaria parasite life cycle stages. We have targeted all enzymes of this pathway for deletion in the mouse malaria model *P. yoelii.* We show that first enzymes of this pathway are dispensable for asexual and sexual blood stage (BS) development but they are essential for mosquito stages development and sporozoite production. However, the last enzymes of this pathway are essential for both BS and mosquito stages development. These results indicate that the first substrates and intermediate products of this pathway can be supplemented by alternative novel pathways inside the blood but not inside the mosquito midgut. Collectively, our data show that CoA de novo biosynthesis is essential for both BS and mosquito stages. This is the first *in vivo* functional characterization of CoA biosynthesis pathway in any protozoan parasite.

1831

A NOVEL RNA APTAMER SYSTEM FOR FUNCTIONAL GENETICS IN *PLASMODIUM FALCIPARUM*

Suresh Maddur Ganesan, Jacquin C. Niles

Massachusetts Institute of Technology, Cambridge, MA, United States

Malaria is a parasitic disease that is widespread in tropical and subtropical regions, and a major cause of human morbidity and mortality. The most severe form of malaria is caused by the parasite, *Plasmodium falciparum*. A limited set of antimalarial drugs is used to treat the disease, but drug resistance is an increasing problem. Hence, identification of novel anti-malarial drugs is a high priority. While our understanding of *Plasmodium* biology has increased in the post-genomic era, tools for doing functional genetics remain quite limited. This impedes progress in identifying key parasite genes and processes that can be prioritized for drug development efforts. To address this need, our laboratory previously developed a novel small molecule-regulated protein-RNA interaction (TetR-aptamer system) that facilitates robust and inducible regulation of target gene translation in eukaryotic organisms including *Plasmodium*. Here, we present the application of protein engineering approaches to integrate our synthetic control system with native *Plasmodium* translational regulatory mechanisms. In so doing, we achieve substantially increased regulatory dynamic ranges (up to 200-fold) compared to a 5-10 fold range of the original system. With a view to identifying new potential drug targets, we are using this system to study several parasite genes. We envision that

this enhancement in regulatory dynamic range will facilitate functional interrogation of larger numbers of parasite genes with greater confidence that associated biological outcomes can be readily identified.

1832

EXPLORING COMPLEX MALARIA INFECTIONS WITH SINGLE GENOME SEQUENCING

Shalini Nair[1], Standwell Nkhoma[2], Karla Gorena[3], Benjamin J. Daniel[3], Francois Nosten[4], Tim J. Anderson[1], **Ian H. Cheeseman**[1]

[1]Texas Biomedical Research Institute, San Antonio, TX, United States, [2]Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Blantyre, Malawi, [3]University of Texas Health Science Center San Antonio, San Antonio, TX, United States, [4]Shoklo Malaria Research Unit, Mae Sot, Thailand

We have recently developed a single cell genomics platform capable of generating whole genome sequence from a individual parasitized red blood cells. This has been extensively validated and can generate SNP calls genome-wide with high (>99%) accuracy, even in species such as *P. vivax* for which long term culture is not possible. This method provides a powerful approach for empirically determining the composition of multiple genotype parasite infections, and provides information that is not accessible using standard illumina sequencing of parasite infections. We have applied this approach to complex malaria infections by sequencing 17 single genomes from multiple-clone infections of *Plasmodium falciparum* (n=1) or *P. vivax* (n=2). After stringent quality control we scored an average of 62,720 *P. vivax* SNPs and 61,080 *P. falciparum* SNPs from each single cell sequence allowing us to map within host divergence between single parasite genomes at exceptional resolution. We use this data to highlight how single cell sequencing can be used to reconstruct genome-wide drug resistance haplotypes from individual infections. Such "phasing" data is expected to be of critical importance for determining the outcome of drug treatment, but cannot currently be determined from bulk sequencing of infections. Second, we have examined the size of blocks of haplotype sharing between genomes within infections and compared these with population data from single clone infections. We observed that parasite genotypes within infections tend to be closely related. Application of single cell genomics and can reveal patterns of relatedness at a fine scale, both within and between malaria infections.

1833

USING GENOMICS TO TRACK PROGRESS TOWARDS MALARIA ELIMINATION

Rachel Daniels[1], Stephen F. Schaffner[2], Noah M. Daniels[3], Wesley Wong[4], Nicholas Baro[4], Hsiao-Han Chang[4], Daouda Ndiaye[5], Daniel E. Neafsey[2], Dyann F. Wirth[4], Sarah K. Volkman[6], Daniel L. Hartl[1]

[1]Harvard University, Cambridge, MA, United States, [2]Broad Institute, Cambridge, MA, United States, [3]Massachusetts Institute of Technology, Cambridge, MA, United States, [4]Harvard School of Public Health, Boston, MA, United States, [5]Cheikh Anta Diop University, Dakar, Senegal, [6]Simmons College, Boston, MA, United States

WHO recommendations for regions of low and moderate endemicity call for program re-orientation at milestones marked by changes in disease prevalence. However, genetic changes in parasite populations may occur before changes in prevalence are measurable. To identify these genomic changes, we examined *Plasmodium falciparum* samples collected from patients in Thiès, Senegal from 2006 to 2013. We genotyped 24 independent SNPs from across the parasite genome - the molecular barcode - to separate annual collections into monogenomic (single parasite genome) and polygenomic (multiple parasite genome) infections. We also performed whole genome sequencing on 190 parasites from monogenomic infections. Following increased control efforts beginning in 2008, we observed large changes in population allele frequencies each season, suggesting enhanced random genetic drift expected from a reduced effective population size. We developed tools to visualize parasite inter-relatedness by molecular barcode and sequencing and identified increasing levels of identity by descent in both. SNP genotyping of monogenomic samples showed clusters with identical molecular barcodes, including several collections where 25-30% of samples shared the same barcode. Whole genome sequence analysis revealed that approximately half of the independent isolates shared between 10 and 98% of their genomes with other sequenced samples, including one obviously hybrid parasite. To our knowledge, this is the first observation of increasing identity by descent in an African population. We show evidence of significant parasite population changes undetectable by standard epidemiological methods. Early identification of population genomic changes associated with changes in transmission offers refined criteria for milestones tracking progress towards elimination. The decreasing costs of genomic analysis make this a feasible option for surveillance of malaria control efforts.

1834

DENGUE VIRUS NONSTRUCTURAL PROTEIN 1 CONTRIBUTES TO VASCULAR LEAK *IN VITRO* AND *IN VIVO*, WHICH CAN BE BLOCKED BY ANTI-NS1 ANTIBODY

P. Robert Beatty, Sarah Killingbeck, Henry Puerta Guardo, Eva Harris

Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States

Dengue virus (DENV) is a mosquito-borne flavivirus consisting of 4 serotypes that causes ~100 million cases of dengue annually. DENV nonstructural protein 1 (NS1) is secreted by infected cells and is found at high levels in patient serum during acute infection. We examined the protective efficacy of NS1 immunization against lethal DENV2 infection in a mouse model of vascular leak. Interferon α/β receptor-deficient C57BL/6 (*Ifnar*$^{-/-}$) mice were injected intraperitoneally 3X over 6 weeks with 20 µg of DENV2 recombinant NS1 (rNS1) combined with different adjuvants, including alum, Sigma adjuvant system (SAS), CpG DNA, Addavax and/or monophosphoryl lipid A (MPLA). Two weeks after the third immunization, mice vaccinated with DENV2 rNS1 with either SAS + CpG or Addavax + MPLA were fully protected against lethal peripheral challenge with DENV2, whereas mice vaccinated with DENV2 rNS1 with alum or CpG DNA alone were not protected. In addition, heterologous cross-protection was observed, as 75% of mice vaccinated with DENV1 NS1 survived lethal DENV-2 challenge. Because NS1 vaccination blocked DENV pathogenesis, we hypothesized that NS1 itself may have direct pathogenic effects. We found that *Ifnar*$^{-/-}$ mice inoculated intravenously with 10 mg/kg of DENV2 NS1 combined with a sublethal dose of DENV2 succumbed 3-4 days post-infection equivalently to mice receiving a lethal dose of DENV2. Mice inoculated with 10 mg/kg DENV2 NS1 alone exhibited morbidity but 100% survived, as did control mice receiving a sublethal dose of DENV2. We then tested the direct toxicity of NS1 on endothelial integrity in a trans-endothelial electrical resistance (TEER) *in vitro* assay. When rNS1 was added to cultured human pulmonary microvascular endothelial cells (HPMEC) in a transwell system, the relative TEER value decreased compared to untreated or OVA-treated HPMEC cells. We next investigated if the *in vivo* lethality and *in vitro* disruption of endothelial integrity caused by rNS1 could be inhibited by NS1-immune serum. *Ifnar*$^{-/-}$ mice passively administered anti-NS1 serum after receiving sublethal DENV2 + NS1 protein were completely protected against death while those receiving control serum were not. In the HPMEC assay, the disruptive effects of rNS1 on TEER were prevented by NS1-immune serum but not serum from OVA-immunized or control mice. Thus, DENV NS1 appears to directly contribute to increased vascular permeability, which can be blocked by anti-NS1 antibody.

1835

DIFFERENCES IN TYPE I INTERFERON SIGNALING ANTAGONISM BY DENGUE VIRUSES IN HUMAN AND NON-HUMAN PRIMATE CELL LINES

Freddy A. Medina[1], Giselle Torres[2], Amanda J. Chase[3], Gilberto A. Santiago[1], Juan F. Medina[1], Luis M. Santiago[1], Jorge L. Muñoz-Jordán[1]

[1]Centers for Disease Control and Prevention, San Juan, PR, United States, [2]University of Puerto Rico Medical Science Campus, San Juan, PR, United States, [3]Mercer University School of Medicine, Macon, GA, United States

The type I interferon (IFN-α/β) response has been shown to be one of the most regulated systems during dengue virus infections. Moreover, attenuation of IFN stimulated genes has been associated with severe disease. In this study we wanted to address conflicting reports of inhibition of IFN signaling by dengue viruses and if there were variations in viruses that differed in their pathogenicity. Using a method that combines flow cytometry and a four-parameter logistic regression model we compared the relative inhibition of IFN-α/β signaling between viruses. Our results showed that all dengue virus serotypes were capable of inhibiting IFN signaling in human cells. A more refined analysis of well-characterized DENV-2 clinical isolates from the five DENV-2 genotypes demonstrated that all viruses inhibited IFN signaling in human cells, but sylvatic viruses displayed a superior ability to inhibit STAT1 phosphorylation. We analyzed inhibition of STAT1 phosphorylation by sylvatic strains in non-human primate cell lines and to our surprise there was no blockage. To determine if these observations were specific to sylvatic strains we performed our IFN inhibition assay with a prototypical DENV2 Asian strain and confirmed that inhibition of STAT1 phosphorylation by dengue viruses does not occur in non-human primate cell lines. However, dengue virus was capable of inhibiting IFN signaling in both human and *Rhesus macaque* primary dendritic cells. IFN-α production was detected in supernatants of dengue virus infected *Rhesus macaque* dendritic cells and contrast to published studies that have suggested that dengue virus can inhibit IFN-α production in human cells. The observed differences in inhibition of the IFN-α/β pathway in human and non-human primate cells may be cell type specific or could result from the transformation process. Nevertheless, these studies provide awareness of differences in the manipulation of the IFN system by dengue virus in human and non-human primate cells.

1836

DENGUE VIRUS NON-STRUCTURAL PROTEIN-1 (NS1) INCREASES HUMAN PULMONARY ENDOTHELIAL CELL PERMEABILITY *IN VITRO*

Henry Puerta Guardo, Dustin Glasner, Eva Harris

Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States

Dengue is the most prevalent arboviral disease in humans and a major public health problem worldwide. Systemic plasma leakage leading to profound shock and potentially fatal complications is a critical determinant of dengue severity. Increased vascular permeability without morphological damage to the capillary endothelium seen in severe dengue suggests the shock syndrome may be due to endothelial dysfunction. In the endothelium, dynamic structures including intercellular junctional proteins and the endothelial glycocalyx control the barrier function critical for vascular homeostasis. In certain diseases, e.g., dengue, functional and structural alterations modify the normal architecture of the vessel wall, increasing plasma extravasation. Dengue pathogenesis involves a complex interaction of the virus and host immune response, including cross-reactive antibodies and T cells, complement activation, and elevated levels of cytokines and other soluble mediators that correlate with severe disease. However, the mechanism of vascular dysfunction in dengue disease is still unclear. Secreted and cell-surface-associated dengue virus nonstructural protein 1 (NS1) and anti-NS1 antibodies are implicated in contradictory roles of protection and pathogenesis, and how NS1 contributes to dengue pathogenesis remains uncertain. Here we evaluated the role of soluble NS1 (sNS1) in inducing endothelial dysfunction. Cultures of human pulmonary microvascular endothelial cells grown on a transwell permeable membrane system as a model of barrier function *in vitro* were exposed to sNS1 (0.2-20 µg/mL), and endothelial permeability was examined by continuously measuring the trans-endothelial electrical resistance (TEER). sNS1 induced a significant dose-dependent increase in endothelial permeability starting 2 hours post-treatment (hpt), at 5 and 20 µg/mL (20 and 50% decrease in TEER, respectively). This effect persisted for more than 24 h as compared to the TEER baseline values exhibited by untreated controls and treatment with unrelated protein (20 µg/mL OVA). Lower concentrations (0.2 and 1 µg/mL) showed less dramatic but still significant decreases in TEER that returned to baseline 6-12 hpt. Confocal microscopy revealed concomitant alterations in intercellular junctional proteins. Our findings suggest a new mechanism of sNS1 directly triggering endothelial vascular dysfunction that occurs in severe dengue disease.

1837

MICROVASCULAR AND ENDOTHELIAL FUNCTION IN PREDICTING CLINICAL OUTCOME OF DENGUE

Sophie Yacoub[1], Cameron Simmons[2], Heiman Wertheim[1], Nguyen Van Kinh[3], Gavin Screaton[4], Bridget Wills[2]

[1]Oxford University Clinical Research Unit, Hanoi, Vietnam, [2]Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam, [3]National Hospital for Tropical Diseases, Hanoi, Vietnam, [4]Imperial College London, London, United Kingdom

Dengue can present with a broad spectrum of clinical phenotypes. The hallmark of severe disease is increased vascular permeability, sometimes leading to hypovolaemic shock. However microvascular/endothelial dysfunction are difficult to assess clinically. A prospective observational study recruiting a) patients presenting to the OPD with fever for <72 hours and a clinical diagnosis of possible dengue, and b) patients hospitalized with warning signs or established severe disease, is ongoing in two hospitals in Hanoi and Ho Chi Minh City, Vietnam. Clinical, laboratory, and haemodynamic assessments are performed daily for a maximum of 6 days, and again at follow-up 2 weeks later. Microvascular imaging using Sidestream Darkfield Imaging (SDF) and endothelial function testing using peripheral artery tonometry (EndoPAT) are performed at enrollment, defervescence/hospital discharge and follow-up. To date, 167 patients have been recruited, 92 in the outpatient arm and 75 in the inpatient arm. The median age is 27 years (range 5-65 years) and 47% are male. In the outpatient arm 29/67 (43%) of the confirmed dengue cases developed warning signs and 3/67 (4%) developed shock, while 25/92 (27%) were diagnosed as having other febrile illnesses (OFI). At enrolment, the reactive hyperaemic index (RHI), a marker of endothelial function, was lowest in the patients who went on to develop severe dengue (median [range] 1. 54 [1.36-1.96]) followed by those who developed warning signs (1.78 [1.17-3.5]) and then uncomplicated dengue (2.18 [1.16-2.29]). In the OFI category the RHI was 1.63 [1.22-3.38]. Results for the inpatient arm showed a similar trend with the lowest RHI seen in severe dengue patients. The SDF images are being analysed; initial results show microvascular flow is impaired in early dengue with a lower proportion of perfused vessels, mean flow index and vessel density compared with follow-up. These preliminary results suggest microvascular and endothelial dysfunction are associated with dengue disease severity, and can be detected prior to severe clinical manifestations. These techniques may prove useful as outcome predictors and/or to monitor endothelium-directed therapies.

1838

MONOCYTE RECRUITMENT TO THE DERMIS AND DIFFERENTIATION TO DENDRITIC CELLS INCREASES THE NUMBERS OF TARGETS FOR EARLY DENGUE VIRUS REPLICATION

Michael A. Schmid, Eva Harris

Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States

The four serotypes of dengue virus (DENV1-4) cause the most prevalent arthropod-borne viral disease in humans. Although *Aedes* mosquitoes transmit DENV via the skin and studies have identified Langerhans cells as targets of DENV replication in the epidermis, no information exists about the immune response and DENV infection in the dermis. DENV suppresses the interferon response, replicates, and causes disease in humans but not in wild-type mice. Here, C57BL/6 mice lacking the interferon-α/β receptor (*Ifnar*$^{-/-}$) had normal frequencies of hematopoietic cells in the skin, were susceptible to intradermal DENV2 infection, and developed disease that displayed key features of severe dengue in humans. For the first time, we identified dermal dendritic cells (DCs), macrophages, and monocytes as targets for DENV replication in the dermis of *Ifnar*$^{-/-}$ mice. We made the following observations. (1) CD103^{+} DCs and macrophages were present in the steady-state dermis and were the first DENV-infected cells in the skin 12 hours post-inoculation (hpi); they then decreased in frequency over time and no longer contributed to DENV replication after 48 h. (2) Substantial numbers of CD11b^{+} Ly6C^{-} DCs were present and were continuously DENV-infected between 12 and 72 hpi. (3) Ly6C^{high} monocytes were actively recruited to the DENV-infected dermis as early as 12 hpi and, by 48 h, differentiated to Ly6C^{+} monocyte-derived DCs (moDCs). Ly6C^{high} monocytes and Ly6C^{+} moDCs became DENV-infected 48-72 hpi and were then the major subsets for DENV replication in the skin. Finally, adoptive transfer of Ly6C^{high} monocytes from *Ifnar*$^{-/-}$ and WT mice confirmed recruitment of circulating monocytes to the DENV-infected dermis, differentiation to Ly6C^{+} moDCs, and DENV infection of *de novo* recruited cells. Our study identifies dermal DCs and macrophages as the initial targets for DENV replication in the skin. Further, we establish a novel mechanism of how DENV exploits the immune response in the dermis by recruiting monocytes and moDCs, which then become the major targets for virus replication in the skin.

1839

DENGUE VIRUS INFECTION INHIBITS THE CGAS/STING/IRF3 PATHWAY IN INFECTED HUMAN CELLS

Sebastian Aguirre[1], Francise Lamote[1], Jenish Patel[2], Ana Maestre[1], Dabeiba Bernal-Rubio[1], Adolfo Garcia-Sastre[2], Ana Fernandez-Sesma[2]

[1]Department of Microbiology and the Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, New York City, NY, United States, [2]Department of Microbiology and the Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai and Icahn Graduate School of Biomedical Sciences at Mount Sinai, New York City, NY, United States

Dengue virus (DENV) has become the most relevant arthropod-borne virus that affects humans. To productively infect the host, DENV needs to inhibit the host innate immune system, particularly the type I interferon (IFN) system. Our group and others have demonstrated that DENV interferes with both, the production and signaling of type I IFN pathways through the expression of viral proteins that specifically target host proteins involved in these essential responses to pathogens. Our group showed that the DENV protease complex NS2B3 is able to interact and cleave the adaptor STING to inhibit the activation of IRF3 in infected cells. A recent report showed an anti flavivirus activity of the newly described pattern recognition receptor cGAS, which after activation generate a second messenger (cGAMP) that in turn activates the adaptor STING and the subsequent induction of type I IFN. In order to investigate the role of cGAS during DENV infection we evaluated the ability of this protein to be activated and trigger type I IFN during DENV infection. We also investigated the role of the NS2B3 protease complex in the inhibition of type I IFN production induced by cGAS. We have found a novel mechanism of type I IFN inhibition by the DENV protease through the interference of the cGAS/STING/IRF3 pathway. Also, over expression of cGAS impaired DENV replication. Alternatively, silencing of cGAS in human dendritic cells (DCs) resulted in a higher accumulation of DENV RNA after infection. These results suggest an active role of cGAS as a sensor during DENV infection and confirm the role of DENV protease as a master regulator of the type I IFN response in DENV infected cells

1840

DEVELOPMENT OF A NON-HUMAN PRIMATE MODEL FOR SECONDARY DENGUE VIRUS INFECTION USING MARMOSETS (*CALLITHRIX JACCHUS*): DETECTION OF VIRUS IMMUNE-COMPLEX USING FCΓ RECEPTOR EXPRESSING CELLS

Meng Ling Moi[1], Kenji Shirai[2], Yasushi Ami[3], Chang-kweng Lim[1], Yuriko Suzaki[3], Kazutaka Kitaura[2], Masayuki Saijo[1], Ryuji Suzuki[2], Ichiro Kurane[4], Tomohiko Takasaki[1]

[1]Department of Virology 1, National Institute of Infectious Diseases, Tokyo, Japan, [2]Department of Rheumatology and Clinical Immunology, Clinical Research Center for Allergy and Rheumatology, Sagamihara National Hospital, National Hospital Organization, Kanagawa, Japan, [3]Division of Experimental Animal Research, National Institute of Infectious Diseases, Tokyo, Japan, [4]National Institute of Infectious Diseases, Tokyo, Japan

Infection with one dengue virus (DENV) serotype does not offer protection against secondary infection against a heterologous serotype. Antibodies to dengue virus (DENV) possess two competing activities: antibody-mediated virus neutralization that leads to protection and infection-enhancement that may cause severe complications. In this study, marmosets (*Callithrix jacchus*) were infected with DENV-2 and subsequently inoculated with DENV-1, DENV-2 or DENV-3 to evaluate the model utility as an infection model for DENV infection. Viremia levels were determined by RT-PCR, BHK and FcγR-expressing cell lines. Antibody response was determined by IgM and IgG ELISA, and neutralizing antibody titers were determined by using BHK cells and FcγR-expressing BHK cells. All marmosets consistently developed viremia after secondary heterologous infection and primary infection. Viremia was absent during secondary homologous challenge. As compared to primary infection, viremia during secondary heterologous challenge persisted longer. Higher levels of viremia were detected using FcγR-expressing cells as compared to FcγR-negative cells during secondary heterologous challenge in marmosets, suggesting presence of infectious virus-immune complex during secondary infection. However, levels of viremia were similar after primary challenge using FcγR-expressing cells and FcγR-negative cells. IgM and IgG antibody response in primary and secondary inoculation were consistent to those of human DENV infection. Marmosets also exhibited thrombocytopenia, leucopenia and increase in AST, ALT and LDH levels during DENV infection. The animal model also demonstrated enlarged liver and kidney during secondary DENV infection. Neutralizing antibodies were serotype cross-reactive in FcγR-negative cells but were specific to primary serotype in FcγR-expressing cells. During secondary infection, marmosets demonstrate viremia and antibody responses consistent with those of human DENV infection. Strong antibody responses induced after secondary heterologous infection possess high neutralizing antibody titers against all four DENV serotypes. The results suggest the potential of marmosets as a useful animal model for DENV infection.

1841

MOSQUITO INFECTIVITY AND GAMETOCYTE CARRIAGE AMONG PATIENTS PRESENTING WITH UNCOMPLICATED FALCIPARUM MALARIA IN NORTHERN CAMBODIA

Jessica T. Lin[1], Ratawan Ubalee[2], Chanthap Lon[2], Michele Spring[2], Sujata Balasubramanian[1], Ponsa Chanudom[2], Namtip Trongnitpatt[2], Worachet Kuntawunginn[2], Anintita Laoboonchai[2], Christian Parobek[1], Nitima Chanarat[2], Char Meng Chuor[3], Jessica Manning[2], Steve Meshnick[1], Charlotte A. Lanteri[2], David L. Saunders[2]

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

Halting the spread of malaria relies on identifying the transmission reservoir and providing effective transmission blocking therapy. Membrane feeding studies have shown that a substantial proportion of *Plasmodium falciparum* infected African children are capable of infecting mosquitoes in the absence of smear-detectable gametocytes. Less is known about transmission in Southeast Asia. We sought to determine the infectivity of adults with *P. falciparum* infection in northern Cambodia relative to gametocytemia. As part of a therapeutic efficacy study of dihydroartemisinin-piperaquine, half of patients with uncomplicated *P. falciparum* were randomized to receive a 45mg single dose primaquine on the 3rd day of dosing. Patient blood was membrane-fed to *Anopheles dirus* mosquitoes prior to treatment and on days 4, 7, and 14 following treatment. At Day 9 after membrane feeding, 50 mosquitoes were dissected for oocyst detection, while another 50 were saved at Days 9 and 16 for parasite detection by real-time PCR. Among 108 patients studied, 7 (6.5%) patients carried smear-detectable gametocytes at baseline (median 66 gametocytes/μL, range 5-728), and only 2 of 7 successfully infected mosquitoes. Both transmitters had high levels of gametocytemia (705 and 728 gametocytes/μL) resulting in high oocyst prevalence (26% and 70% of mosquitoes with average 2 and 56 oocysts/midgut, respectively). Of the remaining patients without smear-detectable gametocytes, only 1 was infectious to mosquitoes, resulting in 8% oocyst prevalence with an average of 1 oocyst/midgut. These results show a 30-fold greater transmission potential in patients with microscopic *P. falciparum* gametocytemia. PCR analysis for submicroscopic gametocytemia and oocyst positivity is currently in progress. However, our findings suggest that in an area with low *P. falciparum* endemicity where gametocyte carriage is relatively rare, only a small minority of patients with symptomatic malaria contribute to the bulk of human-to-mosquito transmission.

1842

THE EFFECT OF ARTEMISININ-COMBINATION THERAPY TREATMENT OPTIONS ON *PLASMODIUM FALCIPARUM* GAMETOCYTE CARRIAGE: A POOLED ANALYSIS OF INDIVIDUAL PATIENT DATA

Teun Bousema[1], Chris Drakeley[2], Philippe Guerin[3], Georgina Humphreys[3], Kasia Stepniewska[3], Carol Sibley[3], Ric Price[3]

[1]Radboud University Medical Center, Nijmegen, Netherlands, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]WorldWide Antimalarial Resistance Network (WWARN) Gametocyte Study Group, Oxford, United Kingdom

The production of gametocytes during malaria is determined by a variety of parasite, human and environmental factors. ACTs rapidly clear the asexual parasite biomass in infected individuals, with potent gametocytocidal activity against early sexual stages of the parasites; they play a critical role in reducing the transmission of malaria and decreasing the spread of drug resistant parasites. We conducted a large pooled analysis of clinical data to examine the differential effect of ACTs on the transmission potential of *Plasmodium falciparum*. A systematic search of the literature was conducted to identify all studies published between 1960 and March 2014, in which patients were enrolled and treated with antimalarials and where gametocyte data were recorded. Individual patients data from over 100 studies (n>40,000 patients) were collated, curated and included in analysis. Data from 21 African and 6 Asian countries was analysed for gametocyte carriage following treatment with artemether-lumefantrine, amodiaquine-artesunate, dihydroartemisinin-piperaquine, and mefloquine-artesunate. An apriori data analysis plan was developed to identify factors associated with gametocyte prevalence and density prior to treatment and following treatment with an ACT. Criteria for the quality of gametocyte assessments have been ascribed to the various studies. In conclusion, the effects of asexual parasite density, age, transmission intensity and haemoglobin concentration on enrolment gametocyte prevalence and density will be presented. The differential effect of ACTs on post-treatment gametocyte carriage, density and carriage time will be examined in relation to ACT regimen, parasite clearance time, transmission intensity and human host factors. The results of this important study and their relevance for malaria elimination will be highlighted.

1843

IVERMECTIN FOR MALARIA CONTROL: INSIGHTS FROM MODELLING

Hannah C. Slater[1], Patrick G. Walker[1], Teun Bousema[2], Azra C. Ghani[1]

[1]Imperial College, London, United Kingdom, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom

Ivermectin (IVM), used alongside mass treatment strategies with an artemisinin combination therapy (ACT), has been suggested as a possible tool for reducing malaria transmission. Mosquitoes feeding on humans that have recently ingested ivermectin have a reduced lifespan, meaning they have a lower probability of completing sporogony and they complete fewer gonotrophic cycles. We use human pharmacokinetic data and mortality data for mosquitoes taking bloodmeals containing IVM to quantify the mosquitocidal effect of IVM. This is incorporated into a transmission model to estimate the impact of IVM in combination with mass treatment strategies with an ACT on transmission metrics. Adding IVM increases the reductions in parasite prevalence achieved and delays the re-emergence of parasites compared to mass treatment alone. This transmission effect is obtained through its effect on vector mortality. IVM effectiveness depends on coverage with the highest impact achieved if given to the whole population rather than only those with existing detectable parasites. Our results suggest that including IVM in a mass treatment strategy can reduce the time taken to interrupt transmission as well as help to achieve transmission interruption in transmission settings in which mass treatment strategies alone would be insufficient. . We also investigate the optimal implementation of ivermectin administration in a range of intervention scenarios, for example whether it best used alongside dihydroartemsinin-piperaquine or artemether lumefantrine in a mass treatment intervention, whether there is any benefit of using primaquine alongside ivermectin, whether ivermectin could be beneficial if used as a stand-alone drug prior to the peak transmission season, and how the vector ecology and existing vector control interventions in a specific region impact the efficacy of ivermectin. Overall, we find that including IVM in mass treatment strategies could be a useful adjunct to reduce and interrupt malaria transmission.

1844

MIND THE GAP: ASSOCIATION BETWEEN HOUSE STRUCTURE AND MALARIA IN UGANDAN CHILDREN

Lucy S. Tusting[1], Humphrey Wanzirah[2], Emmanuel Arinaitwe[2], Agaba Katureebe[2], Kilama Maxwell[2], John Rek[2], Christian Bottomley[1], Sarah Staedke[1], Moses Kamya[3], Grant Dorsey[4], Steve W. Lindsay[5]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Infectious Disease Research Collaboration, Kampala, Uganda, [3]Makerere University College of Health Science, Kampala, Uganda, [4]University of California San Francisco, CA, United States, [5]Durham University, Durham, United Kingdom

Good house construction can lower malaria risk by reducing house entry by vectors. We assessed how house design may affect mosquito house entry and malaria risk in Uganda. 100 households were enrolled in each of three sub-counties: Walukuba, Jinja district; Kihihi, Kanungu district; and Nagongera, Tororo district. Light trap collections were made monthly in all homes. All children aged six months to ten years were followed prospectively to measure parasite prevalence routinely every three months and malaria incidence by passive case detection. Homes were classified as modern (cement, wood or metal walls; and tiled or metal roof; and closed eaves) or traditional (all other homes). We will present the association between house design and human biting rate, malaria infection and clinical malaria and discuss the potential of housing as an intervention against malaria, from low to very high transmission areas.

1845

MAINTAINING UNIVERSAL COVERAGE OF LONG LASTING INSECTICIDAL NETS: IMPACT OF CONTINUOUS DISTRIBUTION ON HOUSEHOLD OWNERSHIP IN EASTERN REGION, GHANA

Celine S. Zegers de Beyl[1], Richmond Ato Selby[2], Obed E. Asamoah[3], Joshua K. Ofori[4], Constance Bart-Plange[5], Hannah Koenker[6], Matthew C. Lynch[6], Kojo Lokko[6], Albert Kilian[7]

[1]Malaria Consortium, London, United Kingdom, [2]Malaria Consortium, Malaria Consortium Kampala, Uganda, [3]Malaria Consortium/NetWorks, Accra, Ghana, [4]NetWorks, Accra, Ghana, [5]National Malaria Control Programme, Accra, Ghana, [6]Center for Communication Programs, Johns Hopkins University, Baltimore, MD, United States, [7]Tropical Health LLP and Malaria Consortium, Montagut, Spain

Distribution of long lasting insecticidal nets (LLIN) is considered a key intervention for the prevention of malaria. Mass distribution is required to rapidly scale up LLIN coverage while continuous distribution systems are essential to sustain the results achieved. In the Eastern Region (ER), the National Malaria Control Programme and implementing partners supported mass LLIN distributions between December 2010 and April 2011. Continuous distribution (CD) activities were started in October 2012 and included antenatal care services), the expanded program on immunization and primary schools. The outcome was evaluated through cross sectional surveys, conducted at baseline in April 2012, 12-16 months after the campaign and at endline in December 2013, after one year of CD implementation. For each survey round, a representative sample of 900 households in ER was selected using a two-stage cluster sampling design. Household heads were interviewed using a structured questionnaire. Household ownership of at least one LLIN was 91.3% (95%CI 88.4 to 93.6) at baseline and fell to 88.4% (85.2 to 91.3) at endline 18 months later but would have been only 81.0% (76.3 to 84.9) without the LLIN from CD. Population access to an LLIN within the household decreased from 74.5% (71.1 to 77.6) at baseline to 66.5% (62.9 to 69.9) but would have been 57.4% (53.0 to 61.8) without the CD contribution. Households reached by any of the CD channels were primarily those who had not been reached by the campaign with any or sufficient ITN. In addition, the different CD channels largely complemented each other with little overlap in the first year. The continuous distribution of LLIN through primary schools and routine health services did not quite maintain the household coverage after one-year of implementation due to its late start almost two years after the campaign. Results show, however, that a CD approach is feasible.

1846

INCREASE IN INTRA-HOUSEHOLD ACCESS TO AND USE OF INSECTICIDE TREATED NETS (ITNS) IN SENEGAL

Julie I. Thwing[1], Adama Faye[2]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Université Cheikh Anta Diop, Dakar, Senegal

With the increase in funding to support malaria control, malaria endemic countries have increased the availability of insecticide treated nets (ITNs) to their populations. Senegal used primarily social marketing until 2008, when a mass distribution campaign targeting children under 5 was conducted, covering 5 of 11 regions. A nationwide campaign targeting children under 5 was conducted in 2009. A rolling universal coverage campaign started in 2010, in which every sleeping space was counted and households received sufficient ITNs to cover every sleeping space, accounting for existing nets in good condition. Between 2010 and early 2013, the universal coverage campaign was implemented in all regions of Senegal. Over this time, 690,000 ITNs were distributed in 2006; 1,065,141 in 2007; 1,586,522 in 2008; 2,532,018 in 2009; 1,258,663 in 2010; 2,465,770 in 2011; and 983,725 in 2012. We used nationally representative survey data to track the evolution of intra-household access to ITNs (a newly recommended indicator), calculated as twice the number of ITNs divided by the number of persons in the household (not to exceed 100%). Based on this indicator, only 11% and 19% of the population had access to an ITN in 2005 and 2006, respectively. In 2008, after the subnational distribution to children under 5 years, access was 36%. The post-campaign survey in 2009 indicated an increase in ITN access to 57%. Universal coverage was completed in four of 14 regions in 2010, resulting in access of 41% nationwide, and 70% in the campaign-covered regions. Access was 63% in 2012, with all but two regions covered. National-level household ownership of at least one ITN from these surveys was 20% (2005), 36% (2006), 60% (2008), 82% (2009), 63% (2010), and 72% (2012), while use by the general population was 6% (2005), 12% (2006), 23% (2008), 34% (2009), 29% (2010), and 41% (2012). Two-thirds of those with access to an ITN reported using it the previous night. Household ITN ownership is an inflated measure of ITN access. Intra-household access is a more appropriate indicator for assessing the gap between ownership and use. Access to nets closely reflects the number of nets distributed annually, and examination of access over time demonstrates the challenge of increasing and maintaining access to ITNs. Additional resources and robust routine distribution strategies are needed to maintain high access to ITNs during the interim periods between larger mass distribution campaigns.

1847

BED NET DURABILITY ASSESSMENTS: EXPLORING A COMPOSITE MEASURE OF NET DAMAGE

Jodi Vanden Eng[1], Ana Paula Abílio[2], Maria do Rosario Pondja[3], Dulcisária Marrenjo[3], Jacinta Luciano[2], Guilhermina Fernandes[3], Samira Sabindy[2], Adam Wolkon[1], Gabriel Ponce de Leon[1], Adeline Chan[1], Juliette Morgan[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Instituto Nacional da Saúde, Maputo, Mozambique, [3]Programa Nacional de Controlo da Malária, Maputo, Mozambique

The durability of Long Lasting Insecticidal Nets (LLINs) in field conditions is of great importance for malaria control programs. Although LLIN bio-efficacy has been investigated, the physical integrity of the net fabric is less well understood making it challenging to determine overall net protectiveness. The 2011 World Health Organization Pesticide Evaluation Scheme (WHOPES) guidelines provide a simple, standardized method using

a proportional hole index (PHI) for assessing net damage. We evaluated the accuracy and utility of this measure using LLINs collected over three years in Nampula Province, Mozambique following a mass distribution campaign in 2008. For each LLIN the type of damage, diameter, and distance from the bottom of the net were recorded for every hole. Holes were classified into four size categories and a PHI was calculated based on the WHOPES guidelines. The areas of WHOPES defined hole size categories were compared to circular and elliptical areas based on actual diameters of each hole; and the PHI was compared to cumulative damaged surface area of the LLIN. The damaged area of small, medium, and large holes was overestimated and the area of extra-large holes was underestimated using the WHOPES categories compared to actual measured areas (Wilcoxon signed rank test of differences p< 0.0001 for all sizes). Approximating holes as circular overestimated hole surface area by roughly 1.5 to 2 times or more. For a range of hypothetical PHI thresholds associated with a "failed LLIN" found in current literature, roughly 75 to 80% of failed LLINs can be detected by only considering large and extra-large holes (which are easier to identify and count). Future research studies may refine the PHI to better approximate overall surface area interrupted. Furthermore, research is needed to identify appropriate PHI thresholds to deem a net no longer protective. Once a cutoff is selected, logistically simpler methods of determining the effective lifespan of LLINs can help guide replacement strategies for malaria control programs.

1848

COMMUNITY ACQUIRED BACTEREMIA AMONG CHILDREN IN AREAS OF LOW AND HIGH MALARIA TRANSMISSION IN RURAL WESTERN KENYA

Frankline M. Onchiri[1], Patricia B. Pavlinac[2], Benson O. Singa[1], Elizabeth A. Odundo[3], Grace C. John-Stewart[4], Judd L. Walson[4]

[1]Kenya Medical Research Institute and University of Washington-Seattle, Nairobi, Kenya, [2]Department of Epidemiology, University of Washington, Seattle, WA, United States, [3]Walter Reed Project, United States Army Medical Research Unit, Kericho, Kenya, [4]Departments of Epidemiology, Global Health and Pediatrics, University of Washington, Seattle, WA, United States

In many African settings, malaria continues to decline as a cause of febrile illness in children, highlighting the need for improved understanding of alternative causes of fever associated with high mortality. We evaluated the prevalence, etiologies, and correlates of bacteremia in outpatient children at two rural hospitals in Western Kenya. Children aged 6 months-15 years presenting consecutively with fever to Kisii hospital; an area of low malaria endemicity (entomological infection rates [EIR]<1.5) and Homa Bay hospital; a malaria endemic area (EIR≥300), were enrolled between 2012 and 2013. Detailed socio-demographic and clinical data were collected and all children tested for malaria using smear microscopy and Paracheck Pf® rapid diagnostic tests, HIV using antibody or PCR testing, and bacteremia using BACTEC™ 9250 blood culture system. Isolates were identified and tested for antibiotic resistance using MicroScan Walkaway40®. Correlates of bacteremia were evaluated using multivariate logistic regression. Overall, 1476 children were enrolled, 742 from Homa Bay and 732 from Kisii. Children from Homa Bay were younger (mean age±SD: 33.9±18.6 vs. 36.8±24.6 months) and more likely to be malaria-infected (49.2% vs. 8.6%), HIV-infected (4.2% vs. 1.2%) or HIV-exposed (19.3% vs. 3.4%) and more severely ill based on presence of ≥1 IMCI danger signs (51.5% vs. 16.9%). Only 48 children (3.3%) had bacteremia (3.1% in Kisii and 3.4% in Homa Bay). Salmonella spp. (19 NTS and 19 typhi) were the predominant cause of bacteremia, accounting for 79.2% (38/48) of all isolates, and the distribution of pathogens did not differ between sites. Bacteremia was associated with HIV infection (aOR=4.5; 95% CI: 1.1-19.3) and lower education of caregiver (aOR=2.6; 95% CI: 1.2-5.7); and inversely associated with malaria infection (aOR=0.4; 95%CI: 0.1-0.9). Bacteremia appears to be a relatively uncommon cause of fever in outpatient children in Western Kenya. Given the infrequent availability of blood culture, targeted testing of high-risk children, including those with HIV, may be a useful strategy to reduce mortality among febrile children.

1849

SALMONELLA TYPHI-SPECIFIC EFFECTOR/MEMORY CD8+ T CELL RESPONSES ELICITED IN A WILD-TYPE *S. TYPHI* CONTROLLED HUMAN INFECTION MODEL

Stephanie Fresnay[1], Monica A. McArthur[1], Thomas C. Darton[2], Claire Jones[2], Claire S. Waddington[2], Christoph J. Blohmke[2], Gordon Dougan[3], Brian Angus[2], Myron M. Levine[1], Andrew J. Pollard[2], Marcelo B. Sztein[1]

[1]University of Maryland, Baltimore, MD, United States, [2]University of Oxford, Oxford, United Kingdom, [3]Wellcome Trust Sanger Institute, Hinxton, United Kingdom

Salmonella enterica serovar Typhi (S. Typhi) is a human restricted pathogen which causes significant morbidity and mortality, particularly in developing countries. A better understanding of the immune responses which result in protection from S. Typhi infection is imperative for the development of improved attenuated vaccines. Current knowledge is limited due to the lack of appropriate clinical and preclinical models. Recently, a controlled human infection model was re-established in which volunteers received 104 colony forming units of wild-type S. Typhi (Quailes strain) orally. Twelve volunteers were evaluated for their cell-mediated immune (CMI) responses. *ex vivo* PBMC isolated before and up to 1 year after challenge were exposed to 3 S. Typhi-infected targets, i.e., autologous B lymphoblastoid cell-lines (B-LCL), autologous blasts and HLA-E restricted AEH B-LCL cells. CMI responses were evaluated using 14-color multiparametric flow cytometry to detect simultaneously 5 intracellular cytokines/chemokines (i.e., IL-17A, IL-2, IFN-γ, TNF-a and MIP-1b) and a marker of degranulation (CD107a). Pre-challenge CD107a expression and cytokine production by S. Typhi-specific CD8+ T effector memory (TEM) following exposure to S. Typhi-infected targets were higher in most volunteers diagnosed with typhoid (TD) compared to those who were not. Direct correlations were observed between the levels of responses before challenge and time to disease onset for CD107a, IFN-g and MIP-1b following stimulation with S. Typhi-infected targets. After challenge, decreases in immune responses were observed prior to the time of disease onset, followed by a sharp increase in most TD volunteers. Multifunctional cells (i.e., concomitantly producing 3-5 cytokines/chemokine and/or expressing CD107a) were dominant at all time-points. These data suggest that S. Typhi-specific responses prior to challenge, as well as the magnitude, kinetics and quality ("multifunctionality") of these responses might play a critical role in the development of typhoid fever.

1850

ACTIVATION OF *SALMONELLA TYPHI*-SPECIFIC REGULATORY T CELLS IS ASSOCIATED WITH TYPHOID DISEASE IN A WILD-TYPE *S. TYPHI* CONTROLLED HUMAN INFECTION MODEL

Monica A. McArthur[1], Stephanie Fresnay[1], Thomas C. Darton[2], Claire Jones[2], Claire S. Waddington[2], Cristoph J. Blohmke[2], Gordon Dougan[3], Brian Angus[2], Myron M. Levine[1], Andrew J. Pollard[2], Marcelo B. Sztein[1]

[1]University of Maryland, Baltimore, MD, United States, [2]University of Oxford, Oxford, United Kingdom, [3]Wellcome Trust Sanger Institute, Hinxton, United Kingdom

Salmonella Typhi (*S.* Typhi), the causative agent of typhoid fever, causes significant morbidity and mortality throughout the world. Currently available vaccines are only moderately immunogenic. To develop improved vaccines, identification of immunological responses associated with protection or disease is necessary. This has been hindered, in part, by the lack of an animal model that faithfully recapitulates human disease. The re-establishment of a controlled human infection model with wild-type *S.* Typhi has made these critical studies possible. Peripheral blood mononuclear cells were obtained from volunteers (n=10) prior to and at multiple time-points after challenge with 10^4 colony forming units of wild-type *S.* Typhi (Quailes strain). Regulatory T cell (T_{reg}) responses were measured by flow cytometry and activation status and homing potential of

S. Typhi-specific T_{reg} were determined. We identified significantly higher gut homing (integrin-a4b7 expressing) *S.* Typhi-specific T_{reg} prior to challenge in volunteers diagnosed with typhoid (TD) than in those who were not (No TD). At early time-points following challenge, circulating integrin-a4b7 expressing *S.* Typhi-specific T_{reg} decreased in TD volunteers, indicating likely homing and a resulting accumulation in the gut. Additionally, *S.* Typhi-specific T_{reg} from TD volunteers demonstrated up-regulation of activation molecules following challenge, including expression of Human Leukocyte Antigen (HLA)-DR and Lymphocyte function-associated antigen (LFA)-1/CD11a as early as 1-4 days post-challenge compared to No TD volunteers. Furthermore, significantly higher expression of the chemokine receptor CXCR3, a molecule associated with homing to sites of active inflammation, was observed on the surface of *S.* Typhi-specific T_{reg} in TD volunteers 1-4 days post-challenge compared to No TD volunteers. Taken together these results suggest that activation of T_{reg} that home to the site of *S.* Typhi infection may play a role in disease pathogenesis, possibly through suppression of *S.* Typhi-specific effector T cell responses.

1851

CHARACTERIZATION OF *CANDIDATUS BARTONELLA ANCASHI*: A NEW AGENT ASSOCIATED WITH CARRION'S DISEASE

Kristin E. Mullins[1], Jun Hang[2], Robert Clifford[2], Fatma Onmus-Leone[2], Ju Jiang[3], Marina Leguia[4], Ciro Maguina[5], Richard G. Jarman[2], David Blazes[1], Allen L. Richards[3]

[1]Uniformed Services University, Bethesda, MD, United States, [2]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [3]Naval Medical Research Center, Silver Spring, MD, United States, [4]U.S. Naval Medical Research Unit - 6, Lima, Peru, [5]Universidad Peruana Cayetano Heredia, Lima, Peru

"The genus" *Bartonella* consists of Gram negative, facultative intracellular, vector-borne bacteria, which infect a wide range of mammalian hosts. *B. bacilliformis*, *B. henselae*, and *B. quintana* have long been recognized as pathogens of human importance, while other species, such as *B. clarridgeiae* and *B. rochalimae,* are newly recognized human pathogens. During a 2003 clinical treatment trial a novel *Bartonella* species, *Candidatus* B. ancashi, was uncovered. This treatment trial was conducted, in Ancash, Peru (where *B. bacilliformis* is endemic), to test the efficacy of azithromycin as a treatment for *B. bacilliformis*. During this trial, two patients were found to be infected with a *Bartonella* species disparate from other *Bartonella* species based on *gltA* sequence typing. Subsequently, this new agent, *Candidatus* B. ancashi, was more completely characterized by 1) observations of *in vitro* microscopic, phenotypic, colonial morphology, and growth characteristics, 2) multilocus sequence typing (MLST), multispacer typing (MST), and whole genome analyses, and by 3) the development of species-specific qPCR assays to identify *Candidatus* B. ancashi's presence in possible vectors (*Lutzoma* spp). Gram-staining and transmission electron microscopy showed the isolates to be small, Gram-negative bacilli with variable expression of unipolar flagella. Biochemical testing provided a single phenotype for all the isolates, which is consistent with other *Bartonella* spp. Fully genome sequencing and subsequent genome analyses confirmed these isolates to be genetically identical to one another, yet distinct from other *Bartonella* species. Genome analyses revealed *B. bacilliformis* to be the closest relative to *Candidatus* B. ancashi, although unlike *B. bacilliformis*, the genome of *Candidatus* B. ancashi encodes virulence determinates not seen in *B. bacilliformis*. Surprisingly, whole genome mapping showed major gene rearrangements between the isolates. Additional genome analyses uncovered a possible link between the rearrangements and flagella expression. Based on the results from these studies, we believe *Candidatus* B. ancashi is a novel pathogen of human importance.

1852

INVESTIGATION OF GENOTYPE VARIATIONS IN *ORIENTIA TSUTSUGAMUSHI* OBTAINED FROM PATIENTS WITH MODERATE AND SEVERE SCRUB TYPHUS

Kristin E. Mullins[1], Daniel Paris[2], Sabine Dittrich[3], Stuart Blacksell[2], Paul Newton[3], Allen L. Richards[4]

[1]Uniformed Services University, Bethesda, MD, United States, [2]Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand, [3]Wellcome Trust–Mahosot Hospital–Oxford Tropical Medicine Research Collaboration, Vientiane, Lao People's Democratic Republic, [4]Naval Medical Research Center, Silver Spring, MD, United States

Orientia tsutsugamushi is a Gram negative, obligate intracellular bacterium that is transmitted by the bite of infected chiggers (*Leptotrombidium* spp) and is the causative agent of scrub typhus. Scrub typhus presentation varies from a mild illness to severe disease, including pneumonitis, meningitis, encephalitis, disseminated intravascular coagulation, and in some cases death. Additionally, *O. tsutsugamushi* is found in Southeast Asia, the southwestern Pacific Islands, Korea, and parts of Russia, China, Japan, Australia, New Zealand, Pakistan, India, and Afghanistan, where over 1 billion persons are at risk for infection and approximately one million are infected annually. While the genus *Orientia* contains only two known species *O. tsutsugamushi* and *O. chuto*, the former is extremely genetically diverse, with >100 genotypes currently recognized. A study is currently underway to examine the relationship between disease severity and genotype. 322 clinical isolates were collected from 6,740 adult patients who presented with suspected scrub typhus in Vientiane (n=4875), Luang Namtha (n=1335), and Salavan (n=530) provinces of the Lao People's Democratic Republic (Lao PDR) from 2004 until 2012. The patients (n=322) were divided by disease severity, with 69 patients exhibiting severe disease and 253 patients exhibiting moderate disease. Severe disease included patients with reduced consciousness (Glasgow Coma Score < 15), shock (systolic pressure < 80 mmHg), jaundice (clinical observations), meningitis/encephalitis (clinical observations), and/or difficulty breathing (respiration rate > 30 breaths/minute). While moderate disease included patients with malaise, fever, headache, and/or rash, who were sick enough to seek medical attention. Additionally, 30 clinical isolates from various locations, within the endemic area for *O. tsutsugamushi,* will be used to improve *O. tsutsugmaushi* genotyping methods. Single Nucleotide Polymorphism (SNPs) analyses will be employed to look for differences between the isolates from various locations in the endemic region and between the isolates that cause severe disease and those isolates that cause a moderate illness in Lao PDR. Through this study, we hope to, identify predictors for severe disease as well as create a more accurate evolutionary phylogeny for *O. tsutsugamushi*.

1853

SPEEDIER *LEPTOSPIRA* DIAGNOSIS USING HEMOCULTURE FLUIDS

Sabine Dittrich[1], Phouthasen Hyongvongsithy[2], Anisone Chanthongthip[2], Michael Knappik[2], Weerawat Phuklia[2], David A. Dance[1], Paul N. Newton[1]

[1]University of Oxford, Vientiane, Lao People's Democratic Republic, [2]Lao-Oxford-Mahosot Hospital Wellcome Trust Research Unit, Vientiane, Lao People's Democratic Republic

Leptospirosis is a common bacterial zoonosis worldwide, with infections occurring after exposure to contaminated water. Despite this global problem, diagnosis is difficult with culture results taking up to three months and Microscopic Agglutination Test (MAT) serology being retrospective by nature. Molecular assays are ~55% sensitive and 90% specific to detect infection on admission blood samples, with low bacterial density complicating detection. Leptospira were shown to survive and multiply in blood culture media and we hypothesised that extracting DNA from incubated hemoculture fluid (HCF) from blood

culture bottles, followed by quantitative real-time PCR (qPCR) could improve the sensitivity and speed of leptospira diagnosis. We assessed this retrospectively, using pre-incubated HCF of leptospira positive (n=109) and negative (n=63) (as determined by culture, PCR directly on clinical samples and MAT on convalescent serum) febrile patients in Vientiane, Lao PDR. After optimization, receiver-operator-characteristics analysis was employed to identify the most suitable qPCR-threshold and corresponding diagnostic values. The finalized method showed promising sensitivities of 82% (95%CI: 71-90), 66% (95%CI: 55-76) and 59% (95%CI: 49-68) compared to culture, culture+PCR or culture+PCR+MAT, as the respective reference standards. The specificities were >95%, for all three comparisons. This approach may enable the diagnosis of leptospiral infection without the submission of additional samples and the incubation step may further increase the sensitivity without compromising the specificity. The optimized protocol and its usefulness in a routine laboratory setting will be further evaluated prospectively during May-October 2014 and these data will be presented.

1854

INNOVATING DIAGNOSIS OF BACTERIAL BLOODSTREAM INFECTIONS IN MALARIA-ENDEMIC SETTINGS: FROM DISEASE METABOLOMICS TO RAPID DIAGNOSTIC TESTS

Saskia Decuypere[1], Jessica Maltha[2], Issa Guiraud[3], Bérenger Kaboré[3], Marc Tahita[3], Stijn Deborggraeve[2], Malcolm McConville[4], Stephen Graham[5], Halidou Tinto[3], Jan Jacobs[2], Jonathan Carapetis[1]

[1]Telethon Kids Institute, Perth, Australia, [2]Institute of Tropical Medicine, Antwerp, Belgium, [3]Clinical Research Unit of Nanoro, Nanoro, Burkina Faso, [4]Metabolomics Australia and Bio21 Institute of Molecular Sciences and Biotechnology, University of Melbourne, Melbourne, Australia, [5]Centre for International Child Health, Melbourne, Australia

The increasing use of malaria rapid diagnostic tests has revealed that febrile illnesses are often caused by other pathogens than *Plasmodium*. Amongst them, bacterial bloodstream infections (bBSI) are increasingly recognized as an important cause associated with a high mortality, especially in the African setting. Currently, diagnosis of bBSI is clinical as microbiological culture testing is usually not available and if available, takes 2 to 3 days for a result. Missed diagnosis can result in preventable deaths, while overdiagnosis results in inappropriate or unnecessary use of antibiotics. There is an urgent need to develop rapid diagnostic tests targeting bBSI. We hypothesize that the pathophysiological processes triggered by bBSI induce characteristic changes in the > 4,000 different blood metabolites. Our objective is to harness these characteristic metabolite features for bBSI diagnosis. We are conducting a first metabolomics study to examine whether blood plasma contains metabolites that could be useful to diagnose bBSI in a malaria-endemic setting. We quantified 1600 polar and lipid metabolites in plasma from 83 children with severe febrile illness admitted to a rural district hospital in Burkina Faso using liquid-chromatography mass-spectrometry. The patients included (i) 12 bBSI cases confirmed by blood culture, (ii) 34 severe malaria cases with a positive thick blood film and (iii) 37 cases with negative blood culture and negative blood film. A distinct metabolite profile was identified in children with culture-confirmed bBSI compared to children with severe malaria. A first diagnostic model including 10 polar metabolites has a sensitivity of 80% (95% CI: 44.4-96.9%) and specificity of 76.5% (95% CI: 62.5-87.2%) to identify culture-confirmed bBSI. Mining of the lipid data is ongoing to fine-tune this diagnostic model for differential diagnosis of bBSI and severe malaria. We will present the predicted diagnosis of the 83 patients by the final metabolite diagnostic model(s), and compare to the results obtained with blood culture, PCR-based SepsiTest™, malaria thick blood smear and HRP2 rapid diagnostic test. This study demonstrates the potential of plasma metabolites to identify causality in children with severe febrile illness. We will discuss the translation to metabolite-based rapid diagnostic tests and their potential impact on clinical management of severe febrile illness in malaria-endemic settings.

1855

MYCOBACTERIUM ULCERANS DISEASE: PERFORMANCE OF DIAGNOSTIC TESTS AND CLINICAL OPINION COMPARED TO DIFFERENT REFERENCE STANDARDS IN AKONOLINGA, CAMEROON

Yolanda K. Mueller[1], Patrick Nkemenang[2], Bitoungui Mboua[3], Sara Eyangoh[4], Genevieve Ehounou[2], Clotilde Rambaud-Althaus[1], Eric Comte[2], Earnest Njih Tabah[5], Jean-Francois Etard[1], Laurence Toutous Trellu[6]

[1]Epicentre, Paris, France, [2]Doctors Without Borders, Geneva, Switzerland, [3]Central Hospital, Yaounde, Cameroon, [4]Centre Pasteur of Cameroon, Yaounde, Cameroon, [5]National Program against Buruli Ulcer, Yaounde, Cameroon, [6]Geneva University Hospitals, Geneva, Switzerland

In the absence of gold standard, the performance of laboratory test to diagnose *Mycobacterium ulcerans* disease (MU) is often overestimated. We compared the diagnostic accuracy of clinical judgment and laboratory tests to diagnose MU. Between 2011 and 2013, all individuals presenting at Akonolinga District Hospital, Cameroon, with a skin lesion suspect of new MU were enrolled after consent. Clinical data and clinicians' judgment on probability of MU (four grades) were prospectively collected, before the results of laboratory examination (ZN, PCR, culture and skin biopsy). Photographs of lesions were reviewed independently by two dermatologists, and skin biopsies by two histopathologists. We constructed a first composite reference standard combining results of laboratory tests, clinical opinion, and final diagnosis reached by expert consensus, and a second based on WHO definition of at least two positive laboratory tests. The 364 included patients had a median age of 34 years (range 0 to 87), 233 (64%) were males and 66 (19%) were HIV-positive. The 364 patients had a total of 422 lesions, of which 381 (90%) were ulcerative. Lesion severity was of category 1, 2 and 3 in 32%, 41% and 26%, respectively. According to expert consensus, MU was diagnosed in 113 (27%) lesions. Main differential diagnoses were vascular ulcers (25%), other bacterial infections (19%), post-traumatic lesions (7%) and non MU osteomyelitis (6%). Area under ROC curve (AUC) for clinical diagnosis compared to consensus reference standard was 0.84 (95CI 0.80 - 0.88), comparable to PCR (0.84, 95CI 0.80 - 0.89, p=0.98), and 0.82 (95CI 0.69 - 0.95) and 0.69 (95CI 0.65 - 0.74) for ZN performed in Akonolinga and Yaounde, respectively. When using a composite standard of two positive tests (pending final culture results for 58/422 lesions), AUC for PCR (0.94 (95CI 0.92 - 0.97) was superior (p<0.001) while clinical judgment and ZN of both sites were comparable (p=0.47). Clinical judgment is at least comparable to ZN to diagnose MU, while PCR is equivalent or superior depending on reference standard used.

1856

OVERCOMING THE CHALLENGES OF CLINICAL DATA MANAGEMENT IN LOW AND MIDDLE-INCOME COUNTRIES: A CONTEXT-ADAPTED DATA MANAGEMENT PLAN AND LIFECYCLE

Yves Claeys[1], James Smedley[2], Anish Bhattarai[3], Hercule Kalonji[4], Paritosh Malaviya[5], Robert Meester[6], David Mwakazanga[7], Martin Omello[8], Sayouba Ouedraogo[9], Sopheak Sok[10], Sun Sokleng[11], Mary Thiongo[12], Hiwot Tilahun[13], Arouna Woukeu[14], Harry van Loen[1], Brian Faragher[2], Raffaella Ravinetto[1]

[1]Institute of Tropical Medicine, Antwerp, Belgium, [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [3]Koirala Institute of Health Sciences, Sunsari, Nepal, [4]University of Kinshasa, Kinshasa, Democratic Republic of the Congo, [5]IMS/BHU, Varanasi, India, [6]Formerly at Amsterdam Institute for Global Health and Development, Amsterdam, Netherlands, [7]Tropical Diseases Research Center, Ndola, Zambia, [8]Interact Program, Uganda, Uganda, [9]CRUN, Nanoro, Burkina Faso, [10]Sihanouk Hospital Center of Hope, Phnom Penh, Cambodia, [11]National Center for HIV/AIDS, Dermatology and STD, Phnom Penh, Cambodia, [12]International Center for Reproductive Health, Mombasa, Kenya, [13]Armauer Hansen Research Institute, Addis Abeba, Ethiopia, [14]Formerly at London School of Tropical Medicine, London, United Kingdom

More and more non-commercial clinical research is carried out on a collaborative basis in resource-limited contexts to address priority health problems in low and middle income countries (LMICs). Despite the existence of FDA- and ICH-GCP guidelines for clinical research, there is a need for a practical approach in all processes including clinical Data Management (DM) to facilitate the collection and analysis of high-quality data, despite the contextual and financial constraints. Since 2010, the members of the Association for Data Management In the Tropics (ADMIT:https://admit.tghn.org) share knowledge and tackle common issues experienced like standardization, Electronic Data Capture and Data sharing. One of the first initiatives for ADMIT was to tackle the need for uniformity in terms of context-adapted standard operating procedures (SOPs).We defined the essential DM processes within a research project. The members of the network were assigned authorship to prepare two SOPs which were presented and peer reviewed during a workshop within the wider group. A harmonization process was undertaken to ensure uniform structure, terminology and the level of detail across the suite of SOPs. During the harmonization, the alignment of the individual processes inspired the creation of an overall lifecycle for DM. As a result, a usable Data Management Plan (DMP) is now available incorporating the suite of these SOP's.(In the oral presentation,) We will present the characteristics of this DMP and describe how it may help with collaborations on non-commercial research projects where DM processes are spread across different places. We recommend the DMP to be used to ensure a uniform approach to DM, strengthening partnerships and knowledge exchange. We hope that this may be a starting point for standardization in DM in LMICs and possibly to formulate practical recommendations for regulatory and GCP guidelines. The next challenge is to look at the definition of the roles and responsibilities needed to resource these DM activities and the development of training packages.

1857

LACTIC ACIDOSIS AND RESPIRATORY DISTRESS ARE FREQUENT IN CEREBRAL MALARIA AND SEVERE MALARIAL ANEMIA, BUT PREDICT MORTALITY ONLY IN CEREBRAL MALARIA

Nathan R. Brand[1], Robert O. Opoka[2], Karen E. Hamre[3], Chandy C. John[3]

[1]Columbia University, College of Physicians and Surgeons, New York, NY, United States, [2]Makerere University, Kampala, Uganda, [3]University of Minnesota, Minneapolis, MN, United States

We investigated the pathogenesis of lactic acidosis (LA) and deep breathing (DB) and their associated mortality in 249 children with cerebral malaria (CM) and 216 children with severe malarial anemia (SMA) in Kampala, Uganda. Platelet count and hemoglobin, lactate and histidine rich protein-2 (HRP-2) levels at admission were assessed. Children with a hemoglobin of <5 g/dL were transfused (all SMA, 59 CM). LA was more frequent in children with SMA (43.1%) than CM (32.1%, P=0.02), and DB was similar in the two groups (CM, 8.8%, SMA, 7.4%, P=0.6), but mortality was higher in children with CM (12.5%) than SMA (0.5%, P<0.001). In children with CM, mortality was increased in children with LA (odds ratio (OR), 2.2, 95% confidence interval (CI), 1.0, 4.7, P=0.04) or DB (OR 5.0, 95% CI, 1.9, 13, P=0.001), but in children with SMA neither LA nor DB was associated with mortality. Children with CM had higher HRP-2 levels and lower platelet counts than children with SMA, while children with SMA had lower hemoglobin levels than children with CM (all P<0.001). In children with CM, both LA and DB were associated with decreased platelet counts and increased HRP-2 levels (all P<0.01), while in children with SMA, LA and DB were not associated with platelet counts, and only LA was associated with increased HRP2 levels (P=0.02). Conversely, hemoglobin levels were inversely associated with lactate levels in children with SMA (P<0.001) but not CM. In children with CM and DB, each natural log increase in HRP-2 levels was associated with an 8.6 fold increased risk of mortality (95% CI, 1.1, 67.0, P=0.04). DB and LA in CM are associated with parasite sequestration with platelet adhesion, while in SMA they are associated with low hemoglobin. These differences may in part explain the high mortality associated with DB and LA in CM, and the lack of DB- or LA-associated mortality in children with SMA who receive a blood transfusion.

1858

WHAT IS AN ASYMPTOMATIC CARRIER IN AN ENDEMIC AREA?

Alejandro Llanos[1], Miguel Campos[2], Mitchel Guzman[3], Roberson Ramirez[3], Raul Chuquiyauri[1], Dionicia Gamboa[3], Hugo Rodriguez[4], Joseph Vinetz[5]

[1]Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Facultad de Ciencias y Filosofia, Universidad Peruana Cayetano Heredia, Lima, Peru, [3]Laboratorio ICEMR-Amazonia, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru, [4]Dirección Regional de Salud de Loreto, Ministry of Health, Loreto, Peru, [5]University of California San Diego, San Diego, CA, United States

Nowadays there is a greater consensus that is crucial to identify asymptomatic carriers in order to eliminate/eradicate malaria. The methods of evaluating clinical malaria in endemic areas, which include both passive (PCD) and active case detection (ACD), are not very effective in detecting asymptomatic infections, limiting treating asymptomatic carriers. Aggressive infection detection (AID) is other strategy where malaria parasites are searched in people of endemic areas, regardless of the presence of clinical symptoms, using PCR and thick smear (TS). However, applied on a large scale AID would be expensive and impractical. In this perspective, we try to understand who is an asymptomatic carrier in an endemic area through an open population cohort study, conducted in the Peruvian Amazon, and involved 2000 people from 8 communities around

Iquitos. AID+ACD and PCD were used. All the subjects were visited once a week in their homes, and they were given a survey where they were asked systematically for 13 malaria-related symptoms, daily recall for the past week. A blood smear for TS was taken weekly and a sample for PCR monthly. It was found that the prevalence of any symptom in the group with PCR(-) and TS(-) was 21.35% (95%CI:20.67-22.43), for PCRPv(+) and TS(-) group was 29.87% (95%CI:26.17-33.40), PCRPv(+) and TS(+) group was 70.5% (95%CI:68.1-72.8) and PCRPf (+) mostly TS(-) group was 25.49% (95%CI:21.48-29.51). Fever was the symptom that best discriminated infection, but it only achieved 60% specificity and 20% sensitivity (40% for the PCRPv(+)/TS(-) group). In addition, 41.4% (95%CI: 38.9- 43.9) of the infections (asymptomatic or no) were negatives to TS. In conclusion, in the Peruvian Amazon study area, 30% of the group with sub-microscopic malaria for *P. vivax* (PCRPv+ and TS-) had at least one symptom. There are statistical differences between the symptom prevalence of PCRPv (+)/TS(-) and PCRPv (+)/TS(+). These results suggest that it is feasible to develop a clinical marker score to detect potential asymptomatic carriers that should be treated.

1859

ANEMIA AND TRANSFUSION REQUIREMENTS AMONG CHILDREN WITH SEVERE MALARIA TREATED WITH ARTESUNATE AT A RESOURCE-POOR HOSPITAL IN UGANDA

Michael Hawkes[1], Andrea Conroy[2], Robyn Elphinstone[2], Sophie Namasopo[3], Chandy John[4], Robert Opoka[5], Kevin C. Kain[2]

[1]University of Alberta, Edmonton, AB, Canada, [2]University of Toronto, Toronto, ON, Canada, [3]Jinja Regional Referral Hospital, Jinja, Uganda, [4]University of Minnesota, Minneapolis, MN, United States, [5]Makerere University, Kampala, Uganda

Treatment of non-immune travelers with malaria using parenteral artesunate is associated with late-onset hemolysis. Most cases of severe malaria occur in children in sub-Saharan Africa, where the hematologic effects of artesunate have not been well documented. We report a prospective case series of 92 children with severe malaria, all treated with parenteral artesunate, managed at a resource-poor hospital in Africa, with detailed longitudinal data on hemoglobin (Hb) levels. The median (range) age was 2 (1-8) years and 43 (47%) were female. Fifteen patients had tea-coloured urine at admission and 14 were visibly jaundiced. The median (IQR) admission Hb level was 69 (56-80) g/L and 17 patients (19%) had severe anemia (Hb<50 g/L). During hospitalization, 69 patients (76%) received one or more transfusions of packed red blood cells or whole blood, for a total of 114 transfusions. The median (IQR) total volume of blood administered was 10.4 (5.6-20) mL/kg. Patients with jaundice at presentation received significantly larger number (p=0.014) and volume (p=0.043) of transfusions. Fatal outcome in 8 patients was associated with severe anemia in 6/8 cases. Follow-up Hb measurement was performed on 35 patients (38%) at day 14 after initial hospital admission; the remaining patients had no clinical evidence of anemia (no pallor, tachycardia, hyperdynamic circulation, parental report of lethargy, or easy fatigability) at the follow-up visit. The convalescent Hb was median (range) 90 (60-138) g/L, which was significantly higher than the paired admission levels (median increase +28 g/L, p<0.001). The day 14 Hb level was higher than any level measured during hospitalization in 22 (63%) patients, but decreased or remained the same in 13 (33%). Among children with a decrease in Hb level by day 14, the magnitude of the Hb change ranged from -8 to -66 g/L, but none reached the threshold for severe anemia (lowest day 14 Hb was 60 g/L). None required transfusion after hospital discharge. In this representative cohort of young children with severe malaria in a hyper-endemic setting treated with artesunate, anemia was common at admission, required one or more transfusions in a majority of patients, and on average was improving by day 14. However, a substantial proportion of children had persistent or worsening anemia at follow-up. Further study is needed to determine whether this effect is attributable to artesunate.

1860

VALIDATION OF A TUBERCULOUS MENINGITIS CASE DEFINITION IN MBARARA REGIONAL REFERRAL HOSPITAL, UGANDA

Susan A. Adakun[1], Emily Evans[2], Bradley Weinbaum[2], Yap Boum II[3], Patrick Orikiriza[3], Mark J. Siedner[4], Tony Wilson[5], Christopher C. Moore[2]

[1]Makerere University College of Health Sciences, Kampala, Uganda, [2]University of Virginia, Charlottesville, VA, United States, [3]MSF Epicentre, Mbarara, Uganda, [4]Massachusetts General Hospital Centre for Global Health, Harvard Medical School, Boston, MA, United States, [5]Mbarara University of Science and Technology, Mbarara, Uganda

Tuberculous meningitis (TBM) is a leading cause of death in areas with a high prevalence of both tuberculosis and HIV such as sub-Saharan Africa. A major obstacle to successful treatment of TBM is the inability to obtain a rapid and accurate diagnosis. We evaluated the accuracy of a recently proposed TBM case definition (Marais, et al) which is based on clinical, CSF, and radiological findings among patients admitted with suspected meningitis to the Mbarara Regional Referral Hospital in Uganda. CSF was obtained for routine analysis, bacterial and mycobacterial culture, and PCR via GeneXpert® MTB/RIF. Blood was obtained for random blood sugar, lactate, malaria blood smear, complete blood count, blood culture, HIV serology and CD4+ count. We determined the diagnostic accuracy for the TBM clinical score by evaluating the sensitivity and specificity, as well as positive and negative predictive value of each score threshold. We used a positive mycobacterial culture of cerebrospinal fluid as a reference standard. We enrolled 141 participants and the prevalence of TBM was 6%. Patients with higher TBM scores were more likely to have a diagnosis of TBM, OR 1.44, p=0.04 CI (1.00-2.06). The ROC curve for the prediction of TBM by the TBM score was 0.75. Of the three case definition criteria (clinical, CSF, evidence of TB), only the CSF criterion was strongly associated with TBM (OR 7.73 95% CI (1.04-57.0) p=0.04). For a TBM score threshold <7, sensitivity was 100%, specificity 38.7%, PPV 7.1%, NPV 100%. The TBM score has good sensitivity but low specificity for the diagnosis of TBM. It has an excellent negative predictive value and may be used to rule out TBM in resource limited settings.

1861

MYOCARDIAL AND HAEMODYNAMIC RESPONSES TO FLUID MANAGEMENT IN SEVERELY MALNOURISHED AND WELL-NOURISHED AFRICAN CHILDREN WITH SEVERE SHOCK AND GASTROENTERITIS

Kathryn Maitland[1], Nchafatso Obonyo[1], Bernadette Brent[2], Peter Olupot Olupot[3], Julius Nteziyaremye[3], Irene Kuipers[4], Michael Boele van Hensbroek[4], Job van Woensel[4]

[1]KEMRI Wellcome Trust Programme, Kilifi, Kenya, [2]Imperial College, London, United Kingdom, [3]Mbale Regional Referral Hospital, Mbale, Uganda, [4]Emma Children's Hospital, Amsterdam, Netherlands

The FEAST (Fluid Expansion as Supportive Therapy) trial, the only randomised controlled trial of fluid resuscitation, demonstrated that fluid boluses lead to excess mortality in children receiving bolus fluids. Further analysis indicated adverse effects of fluid boluses resulted from cardiovascular events rather than fluid overload. Studies of myocardial and haemodynamic responses to boluses are thus warranted in groups not included in FEAST (gastroenteritis and malnutrition), where fluid resuscitation continue to be recommended in international guidelines. We describe in Ugandan children myocardial (echocardiographic and ECG) and haemodynamic responses to fluid bolus (i.e. 0, ½, 1, 1½, 2, 3, 4, 8, 12, 16, 20, 24, 32, 40 and 48 hours) pre- and post fluid challenges recommended by WHO guidelines. Blood and urine samples for analysis of electrolytes and other markers of myocardial dysfunction were collected at admission, 8, 24 and 48 hours. A total of 29 children with severe shock and dehydration (due to gastroenteritis) were studied: 19 had severe malnutrition (SM); 10 were well-nourished (controls). For the

SM group receiving WHO guideline resuscitation (15mls/kg over 1 hour, repeated twice if indicated) mortality was 73% (8/11 patients). Following a protocol amendment to slower rehydration (10mls/kg over 1 hour up to a maximum of 50mls/kg) 3/8 died (38%) compared to a mortality of 2/10 in controls. Echocardiographic and haemodynamic data pre-bolus showed marked evidence of underfilling. Boluses lead to early rapid shock reversal, in those treated with slower rehydration shock reversal was more protracted. In all study participants we found no evidence of that mortality was due to fluid overload. Fluid boluses administered to children with SM (per WHO guideline)-resulted in early shock reversal, but this was not associated a survival benefit. Slow rehydration strategy in cases (SM) and control patients appeared to be well-tolerated. Further research is required to optimize fluid management and other supportive strategies to inform future guidelines.

1862

SEVERE MALARIA INFECTIONS IMPAIR GERMINAL CENTRE REACTIONS AND INHIBIT EFFICIENT ANTIBODY RESPONSES TO INFECTION

Diana S. Hansen, Victoria Ryg-Cornejo, Ann Ly, Lisa J. Ioannidis, Axel Kallies

The Walter and Eliza Hall Institute of Medical Research, Parkville, Australia

Naturally acquired immunity to malaria develops only after many years of repeated exposure to *Plasmodium* parasites. Protective immunity predominantly targets blood-stage parasites and requires antibody responses. Despite the key role that antibodies play in protection against malaria, the cellular processes leading to the slow acquisition of immunity remain unknown. Children in high transmission settings that experience frequent malaria clinical episodes are characterized by a delayed development of parasite-specific memory B cells, suggesting that the inflammatory factors contributing to disease hinder these responses. To address that hypothesis we used a severe malaria infection model to investigate the development of germinal centres (GC), memory B cells and plasma cells. C57BL/6 mice were infected with *P. berghei* ANKA, followed by treatment with anti-malarial drugs or immunized with equivalent antigenic loads of irradiated parasites. Reduced numbers of GC B cells and T follicular helper cells (Tfh) were found in mice experiencing an active infection compared to immunized control animals. Despite normal IL-21 secretion, Tfh cells from infected mice displayed an unusual phenotype characterized by low surface expression of PD-1 and CXCR5, required for their successful localization in GCs. Consistently, confocal microscopy experiments revealed that clinical malaria inhibits the establishment of GC reactions in the spleen. The frequency of memory B cells and relative antibody affinity of long-lived plasma cells emerging from GCs was also examined. Unlike immunization with irradiated parasites, active infections appeared to compromise these processes. Pro-inflammatory cytokines involved in the induction of severe malaria episodes were found be partly responsible for the inhibition of B cell responses. Thus these data indicate that clinical malaria negatively impact the development of long-term humoral immunity by disrupting critical early stages in the development of B cell responses.

1863

ACUTE MALARIA INDUCES CTLA4+PD1+ EFFECTOR T CELLS WITH REGULATORY PHENOTYPE

Maria S. Mackroth[1], Annemieke Abel[2], Christiane Steeg[2], Julian Schulze zur Wiesch[1], Thomas Jacobs[2]

[1]University Hospital Hamburg-Eppendorf, Hamburg, Germany, [2]Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

Acute *Plasmodium falciparum* (Pf) malaria induces proinflammatory T cell responses which have been shown to confer protection against malaria but also contribute to the development of severe disease. A tight regulation of T effector (T_{eff}) responses is therefore crucial to protect the host. An important mechanism to fine-tune T cell responses in the periphery is the induction of co-inhibitory receptors such as CTLA4 and PD1 and their ligands but their role in the immune response in Pf malaria remains poorly understood. To test the hypothesis that co-inhibitory receptors modulate the T cell response in acute malaria, blood samples were obtained from patients with acute uncomplicated Pf malaria treated in Hamburg, Germany as well as from healthy volunteers. Flow cytometric analysis showed high expression of CTLA4 and PD1 on $CD4^+$ T cells of malaria patients and the ligands for PD1, PDL1 and PDL2, were upregulated on monocytes, B cells and T cells. We then stimulated PBMCs with Pf-infected red blood cells (iRBCs) to detect antigen-specific cytokine production and proliferation. The majority of antigen-specific T_{eff} cells were $CTLA4^+PD1^+$. IFNg was the most frequently detected cytokine and >50% of $IFNg^+$ $CTLA4^+PD1^+$ T cells simultaneously produced IL10. In some donors T cell proliferation was inhibited by PD1 and blockade of PD1-ligation enhanced antigen-specific proliferation. We further isolated $CTLA4^+PD1^+CD4^+$ T cells based on surface expression of PD1 and CTLA4 and investigated their inhibitory function in *in-vitro* proliferation assays stimulated with aCD3/28 or iRBCs. $CTLA4^+PD1^+CD4^+$ T cells suppressed aCD3/28-induced as well as plasmodial-antigen-specific T-cell proliferation in a cell-extrinsic manner. In summary, acute Pf infection leads to induction of malaria-specific $CTLA4^+PD1^+T_{eff}$ cells which coproduce IFNg and IL10 while inhibiting $CD4^+$ T cell proliferation in a cell extrinsic manner. Induction of T_{eff} cells with regulatory function might be an important mechanism to control T cell responses and prevent severe inflammation in acute malaria and potentially other acute infections.

1864

LOSS AND DYSFUNCTION OF VΔ2+ ΓΔ T CELLS IS ASSOCIATED WITH CLINICAL TOLERANCE TO MALARIA

Prasanna Jagannathan[1], Charlie C. Kim[1], Bryan Greenhouse[1], Felistas Nankya[2], Katherine Bowen[1], Ijeoma Eccles-James[1], Mary K. Muhindo[2], Emmanuel Arinaitwe[2], Jordan W. Tappero[3], Grant Dorsey[1], Moses R. Kamya[4], Margaret E. Feeney[1]

[1]University of California San Francisco, San Francisco, CA, United States, [2]Infectious Diseases Research Collaboration, Kampala, Uganda, [3]Centers for Diseases Control and Prevention, Atlanta, GA, United States, [4]Makerere University, Kampala, Uganda

The $V\Delta2^+$ subset of γΔ T cells possess intrinsic reactivity to malaria antigens, but their role in acquired immunity to malaria is unclear. To evaluate γΔ T cell responses in children living in a highly malaria-endemic area, peripheral blood mononuclear cells (PBMCs) were obtained from 78 HIV-uninfected 4-year old children enrolled in a longitudinal cohort study in Tororo, Uganda. The incidence of symptomatic malaria in this cohort was 5.4 episodes ppy (IQR 3.2-7.0) and peaked at 25 months of age with a subsequent gradual decline in malaria episodes and a corresponding increase in asymptomatic parasitemia. PBMCs were stimulated with *Plasmodium falciparum*-infected red blood cells (iRBC) or controls and assessed by multiparameter flow cytometry and gene expression microarray. We noted a striking inverse association between frequencies of $V\Delta2^+$ cells and the prior cumulative incidence of malaria (Rho=-0.39, P=0.003). Repeated episodes of malaria were also associated with decreased cytokine production (Rho=-0.41, P=.0002) and decreased proliferation (Rho=-0.58, p=0.009) of $V\Delta2^+$ cells in response to malaria antigen stimulation, suggesting that children who have survived repeated clinical malaria episodes exhibit dysfunction as well as loss of $V\Delta2^+$ cells. Whole transcriptome analysis of sorted, unstimulated $V2^+$ cells revealed increased expression of immunoregulatory genes in children with heavy prior malaria, including genes encoding Tim-3, BATF, and CD57, suggesting that repeated infection may lead to the upregulation of immunoregulatory pathways that dampen the innate V2 inflammatory response. Finally, loss and dysfunction of pro-inflammatory $V\Delta2^+$ γΔ T cells was associated with a reduced likelihood of symptoms upon subsequent *P. falciparum* infection. Together, these results suggest that repeated malaria infection during childhood results in progressive loss and dysfunction of $V\Delta2^+$ γΔ T cells that may facilitate immunological tolerance of the parasite.

1865

LONGITUDINAL ANTIBODY RESPONSES TO ANTIGENS ON THE SURFACE OF *PLASMODIUM FALCIPARUM* GAMETOCYTE-INFECTED ERYTHROCYTES IN GHANAIAN SCHOOL CHILDREN

Bismarck Dinko[1], Teun Bousema[2], Colin J. Sutherland[2]

[1]University of Health and Allied Sciences, Ho, Ghana, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom

Little is known about the immune responses directed at circulating *Plasmodium falciparum* gametocytes in humans, knowledge of which would be useful in the development of intervention strategies to reduce and block malaria transmission. Consequently, antibody responses to surface antigens of *P. falciparum* gametocyte-infected RBCs (GSA) were determined in plasma samples from malaria asymptomatic Ghanaian school children between the ages of 5-17 years. These children were screened for malaria parasites and treated with dihydro-artemisinin piperaquine and followed up weekly for one month. Gametocytes were produced from a laboratory adapted parasite line, 3D7 and a recent patient isolate from Kenya (HL1204). From a cohort of 113 children, 56% of the children exhibited marked antibody responses to GSA (immune response above the median within the cohort per sampling time) that recognized GSA on a proportion of mature gametocyte-infected RBCs of 3D7 by flow cytometry. These responsive individuals were identified by measuring both the proportion of mature gametocytes recognised by antibodies and the intensity of the antibody binding to GSA. Longitudinal data provided an additional 10% developing GSA responses during the 1 month follow-up. Children with GSA antibodies present at enrolment, were less likely to develop new gametocytaemia at subsequent visits (odds ratio = 0.29, 95% CI 0.06 - 1.05; P = 0.034). 3D7a is a laboratory adapted parasite line so a selection of positive plasma samples was tested against mature gametocyte preparations from HL1204 and strong plasma antibody binding was again shown. No binding to the surface of RBCs infected with immature gametocytes of HL1204 was detected. In conclusion, a proportion of malaria infected asymptomatic children harbour plasma antibodies which strongly recognized antigens on the surface of mature gametocyte-infected RBCs. Strong plasma antibody responses were associated with the control of gametocytaemia *in vivo*. Ghanaian GSA responses recognized antigens on both 3D7 and a Kenyan parasite line, suggesting that conserved antigenic determinants are present on the surface of gametocyte-infected erythrocytes.

1866

A SYSTEMATIC CHARACTERIZATION OF MALARIA-ASSOCIATED ATYPICAL MEMORY B CELLS

Silvia Portugal[1], Severin Zinoecker[1], Youssounou Kone[2], Christine E. Schindler[1], Safiatou Doumbo[2], Didier Doumtabe[2], Kishore Kanakabandi[3], Daniel E. Sturdevant[3], Stephen F. Porcella[3], Ogobara K. Doumbo[2], Kassoum Kayentao[2], Aissata Ongoiba[2], Susan Moir[4], Jean-Nicholas Schickel[5], Eric Meffre[5], Boubacar Traore[2], Susan K. Pierce[1], Peter D. Crompton[1]

[1]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [2]Mali International Center of Excellence in Research, Bamako, Mali, [3]RML, National Institutes of Health, Hamilton, MT, United States, [4]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [5]Department of Immunobiology, Yale University School of Medicine, New Haven, CT, United States

Antibodies (Ab) play a critical role in malaria immunity, but Ab-mediated protection is only acquired after years of repeated infections, leaving children in endemic areas vulnerable to repeated bouts of febrile malaria. Many *Plasmodium falciparum* antigens are diverse and clonally variant, contributing to the inefficient acquisition of protective Abs. However, mounting evidence suggests that *Plasmodium*-induced dysregulation of B cell function may also play a role. Several studies have shown that malaria exposure is associated with an expansion of atypical memory B cells (MBCs) which are distinguished from classical MBCs by the expression of inhibitory receptors. A similar subset of B cells has been described in individuals infected with HIV and HCV, yet the origin and function of this B cell subset remains unclear. We performed a comprehensive investigation of atypical B cells collected from individuals exposed to intense malaria in Mali. Sorted naïve B cells ($CD19^+$ $CD21^+$ $CD27^-$), classical MBCs ($CD19^+$ $CD21^+$ $CD27^+$), and atypical MBCs ($CD19^+$ $CD21^-$ $CD27^-$) were subjected to genome wide expression profiling, VDJ sequence analysis (Ab heavy and light chain gene usage and somatic hypermutation rate), KREC analysis (replicative history), as well as proliferative and cytokine production analysis following *in vitro* stimulation. We found that classical and atypical MBCs have distinct expression profiles, but are similar in heavy and light chain variable gene usage as well as replicative history. Atypical MBCs have, however, lower levels of somatic hypermutation in heavy and light chain sequences, indicating less antigen-dependent selection compared to classical MBCs. We further show how these B cell subsets differ in proliferative and cytokine production capacity, and how the expression of inhibitory receptors on atypical MBCs impairs their proliferation. This thorough characterization of B cell subsets in malaria-exposed individuals has generated new hypotheses on how chronic *Plasmodium* exposure leads to B cell dysregulation and the inefficient acquisition of protective antibodies.

1867

CHARACTERIZATION OF MALARIA PARASITE LINES (*PLASMODIUM FALCIPARUM*) SELECTED BY LONG-TERM CULTURE IN THE PRESENCE OF INHIBITORY ANTIBODIES TO APICAL MEMBRANE ANTIGEN-1 (AMA1)

Sheetij Dutta, Lisa Dlugosz, J. Kathleen Moch, Adrian Batchelor, John D. Haynes

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

Vaccination has been the most effective medical intervention (after sanitation, hygiene, and nutrition) for preventing and eliminating infectious diseases. The development of resistance to anti-malarial drugs by the parasite has spurred the search for effective vaccines. It remains unclear, however, if the antigenically highly polymorphic malaria parasite will also evolve resistance to vaccines. Antigenic escape, for example, was observed for Combination B (MSP2) and FMP2.1 (AMA1) malaria vaccines, and the parasite could also shift to alternative invasion pathways that circumvent the requirement of the vaccine antigen. Thus, it is important to study the effects of long term persistence of inhibitory levels of antibodies induced by a *Plasmodium falciparum* blood stage vaccine - using *in vitro* and if possible *in vivo* animal models of malaria. We have recently shown that AMA1 strain-specific antigenic escape could be overcome by inducing broadly inhibitory antibodies using a Quad-allelic formulation of AMA1 (QuadVax, or QV: 3D7+ FVO+HB3+W2mef allelic forms) which elicited high levels of invasion inhibitory antibodies in rabbits against not only all four vaccine strains but also against 22 antigenically diverse non-vaccine strains (Dutta et al. 2013, PLOS Pathogens). We now used anti-QV antibodies to exert immune pressure on two parasite strains (3D7 and W2mef) in long-term cultures. Parasites were maintained in culture for six months in the presence of ~50% inhibitory concentration of anti-QV rabbit serum while the control parasites were maintained in parallel in the absence of antibodies. During the cultures, parasite lines were frozen at various time-points and at the end of the 6 months of culture the parasites were cloned by limiting dilution. Selection pressure was finally removed and anti-AMA1 selected clones were compared to the parental or control selected parasites. Comparative data will be presented regarding (a) growth and invasion rates in the presence or absence of anti-AMA1 antibodies, (b) parasite DNA sequences, (c) invasion into enzyme treated red cells, and (d) quantity and location of AMA1 and its proteolytic processed products. This study informs an important decision point for future development of an AMA1 vaccine as well as malaria blood stage vaccine development in general.

1868

ENHANCED MULTIFUNCTIONAL CD4+ T CELL MEMORY RESPONSES TO MALARIA ANTIGENS IN MALIAN CHILDREN CO-INFECTED WITH *SCHISTOSOMA HAEMATOBIUM*

Kirsten E. Lyke[1], Bourema Kouriba[2], Abdoulaye Dabo[2], Charles Arama[2], Modibo Daou[1], Modibo Daou[2], Issa Diarra[2], Drissa Coulibaly[2], Christopher V. Plowe[1], Ogobara Doumbo[2], Marcelo B. Sztein[1]

[1]University of Maryland, Baltimore, MD, United States, [2]University of Sciences Techniques and Technologies, Bamako, Mali

Durable immunologic memory to malaria is limited in young children, where repetitive and ongoing exposure to malaria is required to achieve and maintain immunity. We have previously demonstrated that schistosomiasis-positive (SP) Malian children, aged 4-8 years, are protected from malaria compared to matched schistosomiasis-negative (SN) children. The effect of concomitant *S. haematobium* upon acquisition of memory to malaria antigens is unknown. We examined antigen-specific T cell frequencies in 48 Malian children aged 4-14 to malaria blood-stage antigens, Apical Membrane Antigen 1 (AMA1) and Merozoite Surface Protein 1 (MSP1) and to schistosoma antigens, Soluble Worm Antigenic Preparation (SWAP) and Schistosoma Egg Antigen (SEA) during a malaria episode and at convalescence 6 months later. CD4+ T cell memory cytokine (IFN-γ, TNFα, IL2 and/or IL17A) responses specific to schistosoma antigens was measured in 18/23 SP children at one or both time points, compared to 4/23 SN children ($p < 0.0001$). At the time of malaria infection, CD4+ T cells from 12/24 SN children and 15/23 SP children ($p=0.29$) stimulated with malaria antigens demonstrated significantly increased levels of cytokine production. In contrast, 7/23 SN children and 16/23 SP children ($p=0.009$) had responses in paired convalescent samples. 46.2% of cytokine-secreting CD4+ T cells expressed a single cytokine after stimulation with malaria antigens during the malaria episode. This fell to 40.9% at follow-up with a compensatory rise of multifunctional cytokine secretion over time (double+: 30.7 to 32.5%, triple+: 20.6 to 23.1%, and quadruple+: 2.4 to 3.8%) consistent with memory maturation. The majority (53.2%-59.5%) of cytokine responses were observed in CD45RA-CD62L- effector memory T cells with little variation depending upon the time point or the study cohort. We conclude that detectable CD4+ T cell memory response can be measured against both malaria and schistosoma antigens and that the presence of *S. haematobium* may be associated with enhanced functional T memory cell induction to malaria antigens.

1869

CAUSES OF MORTALITY IN WOMEN OF REPRODUCTIVE AGE LIVING IN AN URBAN SLUM (KIBERA) NAIROBI

Beatrice Olack[1], Leonard Otieno Cosmas[2], Daniel Ondari Mogeni[1], Joel Mark Montgomery[2]

[1]Kenya Medical Research Institute-Centre for Global Health Research and Centers for Disease for Control and Prevention, Nairobi, Kenya, [2]Global Disease Detection Branch, Division of Global Health Protection, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA USA, Nairobi, Kenya

Women of reproductive age (15-49 years) are confronted by dual burden of health concerns related to maternal conditions: infectious diseases and emerging challenges associated with non-communicable diseases. More than 30% of all deaths in resource-limited settings occur in these women, as compared to 15% in resource-rich settings. There is a paucity of mortality data and causes of death among females of reproductive age in low income countries. We present findings from verbal autopsies among women of reproductive age living in Kibera, an urban slum in Nairobi, Kenya. Verbal autopsies were conducted among women of reproductive age who were participants in a population-based surveillance system and who died between January 2009 and December 2013. Details regarding the death were obtained from close relatives and cause was assigned using the InterVA-4verbal autopsy model (version 4.02). We identified 157 deaths with an overall mortality rate of 5.4 per 1000 person-years of observation. The median age at the time of death was 31.5 years with the highest (40%) proportion of deaths occurring among women 30-39 years of age. Causes of death were identified in 51% of the individuals. Maternal deaths as defined by WHO were less frequent compared to non –maternal deaths (7% vs. 93%, respectively; $\chi^2=80.0$, $p<0.001$) in this population. Among the non-maternal deaths, 62% were due to infectious diseases, with HIV/AIDS associated illness being the leading cause (43%). Non-communicable diseases were associated with 38% of non-maternal deaths, of which cancers and cardiovascular disease were common (43% and 32%, respectively). Communicable diseases were found to be a major cause of death among women of reproductive age; however, non-communicable diseases are increasing in frequency among this population. These findings highlight the need to address and reduce the risk of deaths resulting from both communicable and non-communicable diseases, along with efforts to reduce maternal deaths among women of reproductive age.

1870

REVISITING THE BURDEN OF TYPHOID FEVER IN LOW AND MIDDLE-INCOME COUNTRIES TO INFORM POLICY DECISIONS

Vittal Mogasale, Brian Maskery, R. Leon Ochiai, Jung Seok Lee, Vijayalaxmi V. Mogasale, Enusa Ramani, Young Eun Kim, Jin Kyung Park, Thomas F. Wierzba

International Vaccine Institute, Seoul, Republic of Korea

Typhoid fever still causes significant burden in low and middle income countries where access to safe water and sanitation is compromised. There have been several efforts to quantify the global burden of typhoid fever, but the estimates have not considered the heterogeneity in risk levels within countries. Since the World Health Organization has recommended risk-based use of vaccines against typhoid, we attempted to revisit the burden on typhoid fever in low and middle income countries after adjusting to the risk levels at population. The typhoid disease burden was estimated based on community representative typhoid incidence studies applied to 2010 population after correcting for the operational issues related surveillance, limitations of diagnostic tests and risk difference due to exposure to unimproved water. Incidence estimates, correction factors and mortality estimates were derived from systematic literature review. Scenario analyses for risk factors, blood culture sensitivity and case fatality rates were conducted accounting for the uncertainty in these estimates and compared to previous disease burden estimates. Findings: The risk-adjusted estimate of typhoid fever in low and middle income countries was 11.9 million cases (CI: 9.9 - 14.7 million) and 129,000 deaths (CI: 75,000 - 208,000). In comparison, without the risk- adjustment, the burden estimate would be 20.6 million cases (CI: 17.5 - 24.2 million) with 223,000 deaths (CI: 131,000 - 344,000). Scenario analyses indicated that the risk factor adjustment and updated diagnostic test correction factor derived from systematic literature review were the drivers of difference between current estimate and past estimates. Interpretation: The risk-factor adjusted typhoid fever burden estimate is inherently more conservative than previous estimates that did not account for study site selection bias or fractions of the populations residing in urban slums or rural areas lacking access to improved water supplies. However, by distinguishing and discriminating the risk differences, it allows better estimation of the population level impact and evaluation of cost effectiveness of risk-based vaccination strategies recommended by World Health Organization.

1871

DISABILITY- AND QUALITY-ADJUSTED LIFE YEARS: MEASURING HEALTH OR···?

Thomas Fürst, Maria-Gloria Basáñez, Lesong Conteh

Imperial College London, London, United Kingdom

Disability-adjusted life years (DALYs) and quality-adjusted life years (QALYs) have risen to prominence over the past years and are frequently

used as denominators in cost-effectiveness analyses. They have become a powerful "currency" in health economics, health policy and public health decision making. By combining life years lost due to premature mortality with disability- or quality-adjusted life years reflecting morbidity and attributing these summary measures to specific health conditions and interventions, DALYs and QALYs aim at quantifying health losses and health gains respectively. An explicit assumption is that DALYs and QALYs allow for comparison of different causes of health losses and health gains and that they are therefore suitable to guide global, national and local decision making on where to invest scarce resources. However, based on a literature review, we argue that the DALYs and QALYs lack a clear definition of the concepts "health", "disability" and "health-related quality of life" and therefore also of their disability- and quality-adjustments for individuals' life years spent in less than perfect health. Mainly based on the highly topical International Classification of Functioning, Disability and Health of the World Health Organization, we developed a conceptual framework to delineate what the DALYs and QALYs do, and do not measure. Important similarities and differences between the two measures are revealed. Critical questions about the conceptualization of DALYs and QALYs are discussed and we conclude that if these questions are not addressed there is a continued risk of inefficient decision making and ill-informed advocacy.

1872

VALIDATION OF CAUSES-OF-DEATH USING VERBAL AUTOPSY DATA COLLECTED FROM NAVRONGO HEALTH AND DEMOGRAPHIC SURVEILLANCE SYSTEM IN GHANA: 2007-2011

Patrick O. Ansah[1], Cornelius Debpuur[1], James Akazili[1], Timothy Awine[1], John Williams[1], Nana Akosua Ansah[1], Yazoume Ye[2], Isaac Osei[1], Leopoldo Villegas[2], Osman Sankoh[3], Abraham Oduro[1]

[1]Navrongo Health Research Centre, Navrongo, Ghana, [2]ICFI, Rockville, MD, United States, [3]INDEPTH Network, Accra, Ghana

Determination of cause-specific mortality rate in developing countries is a major difficulty due to poor vital registration systems, deaths occurring outside health facilities, and deaths not being medically certified. Verbal autopsy conducted on these deaths have proven to be one of the reliable methods of compiling cause of death data. We sought to validate the causes of death assigned by physician coders and provide a cause of death structure to improve estimates on cause specific mortality in Ghana. Longitudinal VA data from the Navrongo Health and Demographic Surveillance System (NHDSS) from 2007-2011 was used. Physicians were retrained on death certification and coding using ICD 10 codes and Sample Vital Registration with Verbal Autopsy (SAVVY) methods. VA forms were recoded using the SAVVY methods. In all, 7086 VA forms were retrieved and recoded using SAVVY methods. Males constituted 56% of total deaths and 60% of deaths occurred outside health facilities. The main causes of neonatal deaths were neonatal sepsis (31.5%), birth asphyxia (18.1%) and low birth weight with prematurity (15.4%). Malaria (37.0%), diarrhea (13.1%) and acute respiratory infection (11.6%) were the leading causes of death among children aged 1-11 months. The main causes of death for 1-4 year olds were malaria (53.2%), diarrheal diseases (9.0%), and unspecified infectious diseases (4.7%). Among children 5- 15 years, the main causes of death were malaria (22.5%), accidental drowning, submersions and falls (14.6%) and meningitis (7.7%). For those above 15 years, unspecified non-communicable diseases (14.7%), malaria (6.9%) and cerebro-vascular diseases (6.5%) were the main causes of death in the districts. Variability between two coders using SAVVY method was fair (49.7%; $P<0.001$) with a higher value for neonatal deaths compared to adults. Despite the limitations of VA data the method provides an understanding of the cause of death structure at the population level in developing countries comparable with global estimates that is not possible with existing sources of data.

1873

FALSIFIED MEDICINES IN AFRICA AND PUBLIC HEALTH - 'NO ACTION-TALK ONLY'

Paul Newton[1], Patricia Tabernero[2], Prabha Dwivedi[3], Maria Culzoni[3], Maria Monge[3], Isabel Swamidoss[4], Dallas Mildenhall[5], Michael Green[4], Richard Jähnke[6], Miguel Santos de Oliveira[7], Julia Simao[7], Nicholas White[8], Facundo Fernández[3]

[1]Mahosot Hospital, Vientiane, Lao People's Democratic Republic, [2]University of Oxford, Oxford, United Kingdom, [3]Georgia Institute of Technology, Atlanta, GA, United States, [4]Centers for Disease Control and Prevention, Atlanta, GA, United States, [5]GNS Science, Twin Hut, New Zealand, [6]Global Pharma Health Fund, Frankfurt, Germany, [7]Inspecção Geral de Saúde, Luanda, Angola, [8]Mahidol University, Bangkok, Thailand

Poor quality medicines are neglected impediments to improving global public health. In June 2012 suspected falsified medicines labelled as the antimalarial 'artemether-lumefantrine' bearing the Affordable Medicines Facility-malaria (AMFm) logo and others labelled as the antihelminthic 'mebendazole' were seized in Luanda, Angola. The tablets were analysed by an array of analytical platforms including high performance liquid chromatography, ambient ionization mass spectrometry, Raman spectroscopy, X-ray powder diffraction analysis, nuclear magnetic resonance spectroscopy, isotope-ratio mass spectrometry, botanical assays and packaging analysis, using the portable counterfeit detection device CD-3. No artemether or lumefantrine or other active pharmaceutical ingredients were detected in the 'artemether-lumefantrine' tablets. Brushite and three different yellow dyes and few pollen grains were found. No mebendazole was detected in the 'mebendazole' formulation, but calcite and levamisole (270mg/tablet) were present. Both 'products' showed marked differences in packaging characteristics from genuine products. The discovery of falsified artemether-lumefantrine, labelled as an AMFm product and without any detectable antimalarial, is of considerable concern for malaria control. Presence of levamisole in falsified 'mebendazole' is also of great concern as it has been banned for human use. This seizure illustrates many of the current problems regarding poor reporting and transparency and inaction. Enhanced collaboration between African MRAs/police and the authorities in China to stop criminal transcontinental trade in falsified essential medicines is urgently needed. Delays in reporting and action must be reduced by mandatory notification systems and independent public health risk assessments. Despite multiple reports, public health research has failed to stimulate actions required to improve the quality of global drug supply.

1874

ENHANCING PUBLIC HEALTH RESPONSE TO CLIMATE-SENSITIVE INFECTIOUS DISEASE OUTBREAKS IN FLOOD-PRONE AREAS OF BANGLADESH: ARE PRIMARY HEALTHCARE FACILITIES READY TO RESPOND?

Farhana Haque[1], Mahmudur Rahman[2], Selina Khatun[2], Mujaddeed Ahmed[2], M. Mushtuq Husain[2], Nuzhat Nasreen Banu[2], Md Saiful Islam[3], Md Kamal Hossain[3], Stephen P. Luby[4], Emily S. Gurley[3], James D. Heffelfinger[5]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh and Institute of Epidemiology, Disease Control and Research, Dhaka, Bangladesh, [2]Institute of Epidemiology, Disease Control and Research, Dhaka, Bangladesh, [3]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [4]International Centre for Diarrhoeal Disease Research, Bangladesh and Centre for Infectious Disease and Geographic Medicine, Stanford University, Stanford, CA, United States, Dhaka, Bangladesh, [5]International Centre for Diarrhoeal Disease Research, Bangladesh and Global Disease Detection Branch, Division of Global Health Protection, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, United States, Dhaka, Bangladesh

Global climate change is increasing Bangladesh's vulnerability to natural disasters including floods and raising the potential for infectious

outbreaks. Preparing healthcare for outbreak management is complex and requires comprehensive understanding of the systems' capacities and challenges. We evaluated preparedness of government primary care facilities in flood-prone sub-districts to respond to outbreaks. We asked 69 primary care managers to complete a self-administered survey to assess 7 core capacities critical for outbreak response. We determined percentages of facilities reporting each capacity; assessed managers' perceived preparedness level; and conducted 2 focus group discussions (FGDs) with 24 managers to explore contextual factors driving preparedness. Qualitative data were analyzed using thematic content analysis. All 69 facilities lacked preparedness plans, emergency stockpiles, funds and authority to buy drugs or supplies locally; all lacked dedicated evaluation, isolation and vehicles for infectious patients; 58% lacked sufficient clinicians and 20% lacked adequate field staff. Rapid response teams and digital communication with referral centers existed in all, limited infection control practice in 75%, and diagnostic laboratory in 80% facilities. Unlike lower stockpiling, infrastructure and human resource capacities, higher surveillance, communication, infection control and laboratory capacities were observed. Sixty-three (91%) managers felt their facilities had limited preparedness. There was moderate (71%; Kappa=0.3; P=0.001) correlation between measured and perceived preparedness. FGDs identified insufficient training of providers, low motivation and rapid turnover of clinical staff, and lack of epidemiologists posted at facilities as impediments to human resource capacity. Using a mixed methods approach, this assessment identified deficiencies in all capacities, indicated major gaps in stockpiles, infrastructure and human resource as priority areas for investing constrained resources and offered baseline data for monitoring progress in preparedness. This may be a feasible methodology to evaluate health systems in low-income countries. Development of a contingency plan that secures emergency financing and improves surge capacity by establishing a system to engage existing field workers in outbreaks can sustainably enhance primary care preparedness.

1875

A SYSTEMATIC REVIEW ON THE EFFECTIVENESS OF STRATEGIES TO IMPROVE HEALTH WORKER PERFORMANCE IN LOW- AND MIDDLE-INCOME COUNTRIES: PRELIMINARY RESULTS ON HEALTH OUTCOMES

Alexander K. Rowe[1], Samantha Y. Rowe[1], David H. Peters[2], Kathleen A. Holloway[3], John Chalker[4], Dennis Ross-Degnan[5]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Johns Hopkins University, Baltimore, MD, United States, [3]World Health Organization, New Delhi, India, [4]Management Sciences for Health, Arlington, VA, United States, [5]Harvard Medical School, Boston, MA, United States

Health workers (HWs) play essential roles in delivering health care. In low- and middle-income countries (LMICs), however, HW performance is often inadequate. To characterize the effectiveness of strategies to improve HW performance in LMICs, we conducted a systematic review of 15 electronic databases, 30 document inventories of international organizations, and bibliographies of 510 articles. We included studies meeting accepted criteria for methodological adequacy (e.g., trials with comparison groups) of any strategy on any health topic in any language, published or not. After screening, data from relevant reports were double-abstracted and entered into a database. This analysis focuses on studies that measured health outcomes (morbidity and mortality rates). Effect sizes were calculated as percent change over time in the intervention group minus percent change over time among controls. We screened >105,000 citations, 829 reports met inclusion criteria, and 60 studies measured health outcomes (28 on morbidity only, 24 on mortality only, and 8 with both). Many strategies have been tested, usually with multiple intervention components. The median effect size (MES) across all studies was an improvement of 9 percentage-points (%-points) (interquartile range [IQR]: 0, 39). Among 45 studies focused on facility-based HWs, the strategy with the greatest health impact was HW training + group problem solving (MES = 49 %-points, IQR: 24, 77). Often used strategies, such as HW training and supervision, alone or in combination, had lower effect sizes (typically ranging from no effect to +16 %-points). Among 15 studies focused only on community HWs, the strategy with the greatest health impact was consumer supports (e.g., patient education) + HW training + providing drugs or equipment (MES = 55 %-points, IQR: 15, 62). Contextual and methodological heterogeneity made comparisons difficult. Results from this review, which will be finalized by the end of 2014, should inform decision-making on how best to improve HW performance and health outcomes in LMICs.

1876

PREVALENCE OF TRACHOMA IN BRAZILIAN SCHOOLCHILDREN

Expedito J. Luna[1], Maria F. Lopes[2], Norma H. Medina[3], Maria R. Cardoso[1]

[1]Universidade de Sao Paulo, Sao Paulo, Brazil, [2]Ministerio da Saude, Brasilia, Brazil, [3]Secretaria Estadual de Saude de Sao Paulo, Sao Paulo, Brazil

The aim of the study was to estimate the prevalence and describe the distribution of trachoma among schoolchildren in Brazil. We conducted a cross-sectional study, using cluster sampling of the schoolchildren population, living in Brazilian municipalities with Human Development Index lower than the national mean. This prevalence survey was conducted by the Brazilian Ministry of Health, in the period 2002-2008. 3,144 schools, with 176,224 schoolchildren, from 1st to 4th grades, located in 1,491 municipalities, were selected. The selected schoolchildren underwent an external ocular examination, with a magnifying glass (2.5X), to detect clinical signs of trachoma according to the WHO grading criteria. The prevalence of trachoma, by state and national level, and their respective 95% confidence intervals were estimated. Chi-square and chi-square for trends tests were used to compare categorical variables. 8,526 cases of trachoma were detected, resulting in a prevalence of 5.00% (95%IC 5.05; 4.95). Most cases were mild (TF prevalence = 4.92%). Prevalence of intense inflammatory trachoma (TI) and trachomatous scarring (TS) was low: 0.03% and 0.05% respectively. There was no significant difference between the sexes. The prevalence of trachoma was 10.8% among children under 5 years of age, decreasing as age increased (chi square for trend $p < 0.00001$). There was a significant difference in prevalence between urban and rural areas, 4.3% *versus* 6.2% respectively ($p < 0.001$). Cases were detected in 1,189 municipalities (80% of the municipalities in the sample), in all 27 states of the country. In 37% of the selected municipalities, the prevalence was higher than 5%. The study has shown that trachoma is still endemic in a large proportion of the poorer Brazilian municipalities, contradicting the belief that the disease had been controlled in the country. The survey provided a baseline for evaluating planned interventions aimed at achieving the goal of certification of elimination of trachoma as a cause of blindness in Brazil by 2020.

1877

BACTERIAL LOAD AND PATHOGEN DIVERSITY IN OCULAR INFECTION WITH *CHLAMYDIA TRACHOMATIS* IN A TRACHOMA-HYPERENDEMIC ISLAND SETTING

Anna Last[1], Fengchen Liu[2], Travis Porco[2], Chrissy Roberts[1], Eunice Cassama[3], Meno Nabicassa[3], Sarah Burr[4], Taane Clark[1], Francesc Coll[1], Nick Thomson[5], Thomas Lietman[2], Martin Holland[1], Robin Bailey[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]University of California San Francisco, San Francisco, CA, United States, [3]Ministerio de Saude Publica, Bissau, Guinea-Bissau, [4]Medical Research Council The Gambia Unit, Fajara, Gambia, [5]Wellcome Trust Sanger Institute, Cambridge, United Kingdom

Trachoma is caused by ocular infection with *Chlamydia trachomatis (Ct)*. Acute conjunctival infection may recur and progress to a chronic inflammatory process causing conjunctival scarring and blindness. The

reasons why active and scarring trachoma are so prevalent on the Bijagós Archipelago of Guinea Bissau are unclear. We collected 1507 population-based conjunctival swabs with corresponding detailed clinical phenotype. We used droplet digital PCR assay to detect and quantitate *Ct* DNA on swabs. Associations between *Ct* load and clinical phenotype were examined using regression models. We used agent-based modeling to investigate the role of *Ct* load in trachoma transmission. Whole genome sequence analysis was used to identify variants in putative virulence-associated genes/loci. The geometric mean of estimated *Ct* load in clinically normal conjunctivae was 294 copies/swab (95% C.I. 165-524). In clinically active trachoma it was 8562 copies/swab (95% C.I. 5412-13546). In active trachoma *Ct* load increases with disease severity (for both follicular and inflammatory scores). The highest *Ct* loads were associated with the most severe clinical disease and the strongest associations were with increasing inflammatory grade (at maximal inflammatory score (P3) OR 30.9, 95% CI 9.39-101.5, *p<0.0001*). Genotypic differences in virulence-associated genes within this population of ocular *Ct* are suggested. The association between load and disease severity may be related to *Ct* strain diversity, where multiple strains are co-circulating. We used a novel mathematical modeling strategy to investigate the role of *Ct* load in trachoma transmission. This is the first application of these approaches in understanding the pathogenesis and transmission of *Ct* infection, which are fundamental to successful trachoma elimination and surveillance strategies.

1878

ASSESSING THE BURDEN OF PEDIATRIC ACUTE RHEUMATIC FEVER AND RHEUMATIC HEART DISEASE --- AMERICAN SAMOA, 2011-2012

Amanda L. Beaudoin[1], Laura Edison[1], Camille E. Introcaso[2], Lucy M. Goh[3], Chris Van Beneden[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Pennsylvania Hospital, Pennsylvania Center for Dermatology, Philadelphia, PA, United States, [3]Lyndon Baines Johnson Tropical Medical Center, Pago Pago, American Samoa

In August 2013, LBJ Tropical Medical Center and the American Samoa (AS) health department notified CDC of a perceived high burden of pediatric acute rheumatic fever (ARF) and rheumatic heart disease (RHD). ARF is an immunologically mediated sequela of inadequately treated group A *Streptococcus* pharyngitis and, potentially, pyoderma. Recurrent or severe ARF can cause permanent cardiac damage and RHD. Long-term prophylactic penicillin injections post-ARF diagnosis can prevent RHD. We aimed to describe pediatric ARF and RHD and prophylaxis in AS and the pyoderma-ARF association. We used ICD-9 codes and hospital prophylaxis registries from AS's only medical system to identify all patients aged ≤18 years with a physician-recorded ARF or RHD diagnosis during 2011-2012. We recorded penicillin compliance and pre-ARF pharyngitis and pyoderma diagnoses (≤6weeks preceding) for cases. Two age- and sex-matched control subjects per case-patient were selected from non-ARF/RHD patients examined during 2011-2012. We calculated ARF 2011-2012 incidence and RHD prevalence by using 2010 U.S. Census data. We used univariate statistical tests and conditional logistic regression for case-control comparisons. During 2013, RHD prevalence was 3.2 cases/1,000 children. ARF incidence was 1.1 (2011) and 1.5 (2012) cases/1,000. Of 65 children diagnosed with ARF during 2011-2012, a total of 32 (49%) subsequently received RHD diagnoses. Median ARF diagnosis age was 11 (range: 2-18) years. Pharyngitis history was more common among case-patients (18%) than control subjects (0%; P <0.01), but preceding pyoderma was not. Post-ARF penicillin prophylaxis compliance (65%) was suboptimal. RHD causes considerable childhood morbidity in AS. Although the pyoderma-ARF association remains unclear, attempts to curb AS's RHD burden should address improved pharyngitis diagnosis and treatment and increased ARF prophylaxis compliance.

1879

REPRODUCTIVE TRACT INFECTIONS AMONG PRIMARY SCHOOLGIRLS IN RURAL WESTERN KENYA

Newton Otecko[1], Emily Kerubo[1], Elizabeth Nyothach[1], Alex Morwabe[1], Harrison Fredrick[1], Kelvin Oruko[1], Clifford Oduor[1], Preston Owiti[1], John Vulule[1], Frank Odhiambo[1], Kayla Laserson[2], Clement Zeh[2], Penelope Phillips-Howard[3]

[1]Kenya Medical Research Institute-Centers for Disease Control and Prevention Research and Public Health Collaboration, Kisumu, Kenya, [2]Centers for Disease Control and Prevention, Kisumu, Kenya, [3]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Reproductive tract infections (RTIs) among adolescent girls remain a great public health concern in developing countries. However, the burden of RTIs in this key population is insufficiently understood. This study presents preliminary analysis of RTIs' symptoms reporting and laboratory detection rates among girls aged 14-16 (median 14) years, in 30 primary schools, enrolled in a feasibility study on acceptance, use and safety of menstrual products in western Kenya. Vaginal self-swabbing samples were prospectively collected during symptom guided RTI testing (SGT) (March-October 2013) and cross-sectional end-of-study screening (EOSS) (November 2013). Samples were analyzed for Bacterial vaginosis (BV), Chlamydia trachomatis (CT), Neisseria gonorrhea (NG), Trichomonas vaginalis (TV) and Candidiasis. Infected girls were referred for treatment. Data were analyzed using SPSS v.21.0. Overall, 532 girls (SGT: 17, 3.2%; EOSS: 453, 85.2%; and overlap in both: 62, 11.7%) were included. Of a total 79 girls in SGT group, BV 13 (16.5%), Candidiasis 11 (13.9%), TV 5 (6.3%) and CT 2 (2.5%) were test confirmed. None tested positive for NG. BV was the most common in EOSS, 94 (18.3%), followed by Candidiasis 44 (8.5%), TV 13 (2.5%), CT 13 (2.5%) and NG 3 (0.6%). Of 62 girls in both STG and EOSS, RTI detection rates varied (SGT-EOSS) for BV (19.4%-14.5%), Candidiasis (14.5%-12.9%), TV (6.5%-0%), CT remained constant. While only 82 (15.9%) girls reported symptoms for RTI at EOSS, laboratory testing showed 146 (28.3%) had at least one RTI. In conclusion, high detection rate of RTIs was observed among the rural adolescent schoolgirls. Symptom-based diagnosis of RTIs poorly predicted RTI in this population. These findings offer important insights for treatment and prevention of RTIs among schoolgirls.

1880

ACCURACY OF THE INTEGRATED MANAGEMENT OF CHILDHOOD ILLNESS (IMCI) ALGORITHM IN IDENTIFYING CULTURE-CONFIRMED DIARRHEAL PATHOGENS REQUIRING ANTIBIOTIC THERAPY

Patricia B. Pavlinac[1], Frankline M. Onchiri[1], Grace C. John-Stewart[1], Jaqueline M. Naulikha[1], Denno M. Donna[1], Elizabeth A. Odundo[2], Benson O. Singa[3], Lisa E. Manhart[1], Judd L. Walson[1]

[1]University of Washington, Seattle, WA, United States, [2]United States Army Medical Research Unit, Kericho, Kenya, [3]Kenya Medical Research Institute, Nairobi, Kenya

Diarrhea is the second leading cause of death in children under 5, with most deaths occurring in settings where microbiology facilities are unavailable or cost prohibitive. The World Health Organization (WHO) developed the Integrated Management of Childhood Illness (IMCI) guidelines to manage sick children based on clinical signs and history. For children with diarrhea, the guidelines recommend empiric antibiotics for children with suspected shigellosis (presence or history of bloody stool) or suspected cholera (age ≥2 years, severe dehydration, and living in a cholera endemic area). We assessed the diagnostic performance of the IMCI guidelines for diarrhea management as compared to stool bacterial culture. Children aged 6 months to 5 years presenting to two Western Kenya District hospitals between December 2011 and September 2013 with acute diarrhea were enrolled. Stool samples were tested using standard methods for bacterial culture. Multiplex PCR was used to further classify diarrheagenic *Escherichia coli*. Among 973 enrolled children,

median age was 17 months (interquartile range 10-34), 16.5% were stunted, and 4.4% were HIV-infected. The most predominate bacterial isolate was EAEC (14.1%), followed by *Campylobacter* (6.6%), EPEC (6.2%), *Shigella* (4.6%), and ETEC (4.4%). IMCI correctly classified 3 of 45 lab-confirmed *Shigella* cases (sensitivity 6.7%), 2 cases of *Shigella flexneri* and 1 *S. dysenteriae*. Among 928 children without shigellosis, IMCI correctly classified 871 (specificity 93.9%). Of the 57 children incorrectly diagnosed with *Shigella* by IMCI, 73.7% had no other bacterial pathogen identified. Cholera was not detected although 11 (1.1%) children were classified as having suspected cholera based on IMCI criteria (specificity 98.9%); of these 11, 36.4% had no isolated bacteria. The IMCI guidelines appear reasonably specific but not sensitive in identifying children requiring antibiotic therapy. IMCI guidelines should be adapted to enhance sensitivity and to account for additional enteric pathogens associated with increased morbidity and mortality.

1881

EVALUATION OF INTEGRATED MANAGEMENT OF ADOLESCENT AND ADULT ILLNESS DISTRICT CLINICIAN MANUAL EMPIRIC ANTIMICROBIAL THERAPY RECOMMENDATIONS FOR SEVERE INFECTIONS IN NORTHERN TANZANIA

Matthew P. Rubach[1], Venance P. Maro[2], John A. Bartlett[1], John A. Crump[1]

[1]Duke University, Durham, NC, United States, [2]Kilimanjaro Christian Medical Centre, Moshi, United Republic of Tanzania

We assessed the effectiveness of Integrated Management of Adolescent and Adult Illness District Clinician Manual (IMAI) empiric antimicrobial therapy recommendations for septic shock, severe respiratory distress without shock, and severe pneumonia in hospital settings in northern Tanzania. IMAI recommended empiric therapies were retrospectively evaluated against laboratory-confirmed etiology of illness data for participants in a febrile illness cohort study who met IMAI criteria for the three clinical syndromes. Therapies evaluated included IMAI emergency antibacterials (ceftriaxone or ampicillin plus gentamicin) for septic shock and severe respiratory distress without shock, and ceftriaxone plus a macrolide for severe pneumonia. Among 423 participants hospitalized with febrile illness, 171 cases met IMAI criteria for the three syndromes: 25 septic shock, 37 severe respiratory distress without shock, and 109 severe pneumonia. Forty-four (10%) of 423 participants died in-hospital. Ceftriaxone was the single-most effective agent in all three syndromes, being effective for 12 (48%) septic shock, 5 (14%) severe respiratory distress without shock, and 18 (17%) severe pneumonia illnesses. For each syndrome 17-27% of participants had an etiologic diagnosis non-responsive to ceftriaxone, but responsive to other available antimicrobial regimens, namely amphotericin for cryptococcosis and histoplasmosis; anti-tuberculosis therapy for bacteremic disseminated tuberculosis; or tetracycline therapy for rickettsioses and Q fever. IMAI recommendations for empiric ceftriaxone to treat septic shock, severe respiratory distress without shock, and severe pneumonia are warranted. Etiologies not explicitly addressed in IMAI guidance for these syndromes, such as cryptococcosis, histoplasmosis and tetracycline-responsive bacterial infections, were common. Prospective assessments of IMAI are needed to confirm these results and improve syndromic management algorithms.

1882

IMPACT OF FUTURE CLIMATIC CONDITIONS ON VIRAL, BACTERIAL AND PROTOZOAN ENTERIC PATHOGENS

Karen Levy, Sharia M. Ahmed, Rebecca Philipsborn, Lily M. Tyndall, Berry J. Brosi

Emory University, Atlanta, GA, United States

Understanding the seasonality of infectious diseases is important, in order to prepare for case loads, plan vaccination campaigns, and to anticipate impacts of climate change. Diarrheal diseases are often cited as one of the major health impacts of climate change, but there much uncertainty remains in the estimates associated with the relationship between climatic drivers and diarrheal disease. One source of this uncertainty is the variety of pathogens associated with infectious diarrhea, each of which has different life cycle characteristics such as survival outside of the host. Therefore it is important to understand how the seasonality of different etiological agents of diarrheal disease vary. In this systematic review and meta-analysis, we examined the impact of climatic variability on three representative diarrheal disease pathogens of different taxa: pathogenic E. coli, norovirus, and cryptosporidium. Incidence data for each pathogen were taken directly from tables or extracted from graphs in the published papers. Year-specific monthly temperature and precipitation from each location at the time of disease data collection were assembled from publicly available datasets. We examined the relationship between climatic variables and incidence of each pathogen for each location using generalized log-linear Poisson regression models, and we also pooled all datasets for each pathogen to calculate an overall association between monthly cases and mean monthly temperature, using a generalized estimating equation. We then used the model results to examine what proportion of the total of cases attributable to these three pathogens would be attributable to any one of the pathogens given increases in temperature of 1-4°C, in increments of one degree. We found that a one-unit increase in temperature was associated with increases in incidence of pathogenic E. coli (IRR = 1.08, 95% CI = 1.04-1.11) and Cryptosporidium (IRR=1.03, 95% CI = 1.03-1.04) and decreases in Noroviurs (IRR = 0.92, 95% CI: 0.90-0.94). These results highlight the importance examining taxa-specific climate-disease relationships for enteric diseases. As temperatures increase under future warming scenarios, bacterial and protozoan pathogens are expected to represent an increasingly large fraction of the burden of diarrheal disease. This has important implications for development of control strategies.

1883

FLY ME TO THE PLUME: VIDEO-TRACKING ANALYSIS OF *ANOPHELES GAMBIAE* FLIGHT BEHAVIOR AT HUMAN-BAITED BEDNETS

Josephine Parker[1], Natalia Angarita-Jaimes[2], Mayumi Abe[1], Fabian Mashauri[3], Jackline Martine[3], Catherine E. Towers[2], David Towers[2], Philip J. McCall[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]Optical Engineering Group, School of Engineering, University of Warwick, Coventry, United Kingdom, [3]National Institute for Medical Research, Mwanza, United Republic of Tanzania

Understanding how mosquitoes interact with insecticide-treated bednets (LLINs) is fundamental to advancing the design and performance of LLINs, and to ensuring they continue to be effective and sustainable tools for malaria prevention. We have developed an innovative video system that enables nocturnal flight activity of multiple mosquitoes at a human occupied bednet to be captured at high resolution, and individual mosquito flight paths and details of movements to be tracked and analysed over periods of 60 minutes or more. Following initial laboratory studies, the system has been deployed in an experimental hut at a field site in Tanzania, where we are investigating behaviour of local *Anopheles* sp. populations entering the hut in response to human baits in untreated and insecticide-treated bednets. Analysing flight tracks, we have classified mosquito activity into four broad types, termed 'swooping', 'visiting', 'bouncing' and 'resting'. Mosquitoes flew more slowly and flight paths were more tortuous when nets were baited. Responding to human bait, most activity was spent in flight. The majority of contacts made with the net surface were very brief (duration less than 4 seconds) and activity occurred primarily on the top surface of the net over the sleeper's torso, with less activity seen at the supine human's feet. This finding is consistent with previous studies suggesting that hostseeking mosquitoes orient towards a 'plume' of host attractants, funnelled upwards by the 'chimney' effect of the bednet walls. We compared activity on untreated nets with Permanet 2.0 (deltamethrin-treated LLINs) to examine how treatment

influenced mosquito behaviour. Results investigating flight patterns and visiting patterns at the net surfaces, changes in activity patterns over time and LLIN repellency will be presented and implications for current and future LLIN-based approaches will be considered.

1884

SWARMING BEHAVIOR OF *ANOPHELES GAMBIAE* MALES INCREASES FEMALE INSEMINATION RATE IN CAGED POPULATIONS: AN OPPORTUNITY TO STUDY MOSQUITO MATING SYSTEMS

Luca Facchinelli[1], Laura Valerio[1], Clelia Oliva[2], Rosemary S. Lee[2], Tania Persampieri[1], C. Matilda Collins[3], Andrea Crisanti[1], Roberta Spaccapelo[1], Mark Q. Benedict[1]

[1]University of Perugia, Perugia, Italy, [2]Polo d'Innovazione Genomica, Genetica e Biologia S.C.a.R.L., Perugia, Italy, [3]Imperial College London, London, United Kingdom

Long-term control of *Anopheles gambiae*, the major malaria vector in Africa, is difficult to achieve and maintain with traditional control methods. Genetically Modified (GM) *A. gambiae* strains that bear sterility genes are potentially powerful new tools to control the disease. Natural male mating behavior is a key component in GM mosquito control strategies in order to spread transgenes into the wild type population. Mating behavior involves the ability of males to form swarms, which is crucial to inseminate females in the wild. In spite of its biological importance and relevance for vector control, swarming is a poorly understood process because it is hard to study in the wild and difficult to stimulate under laboratory conditions. Here we describe features that promote male *A. gambiae* G3 strain swarming in large cages. In 15.6 m3 cages, a dark foreground and contrasting illuminated background with a contrasting mark on the ground stimulated swarm formation during artificial twilight. G3 males have not lost their capability to swarm although this strain has been colonized since 1975. We asked whether swarming behavior would affect mating performance of wild-type (WT) G3 and I-PpoI transgenic *A. gambiae* sexually sterile males competing for G3 females. We performed competitive matings and recorded female insemination rate and proportion of matings by WT and GM males. The presence of swarming stimuli was associated with an increase in mating frequency from 77.4 to 97.4 %. There was no change in competitiveness by transgenic males as a function of swarming stimuli. The increase of mating frequency in the presence of swarming stimuli highlights the importance of swarming in *A. gambiae* mating behavior. Reproducing *A. gambiae* swarms in controlled conditions provides the possibility to dissect the mating behavior of this species and explain the mechanisms controlling it, which is innovative in mosquito research. We will discuss the results and the possible applications of our findings to investigation of *A. gambiae* biology and to support vector control strategies

1885

NOVEL INSIGHTS INTO GENETIC CONTROL USING EXPERIMENTAL AND MATHEMATICAL SIMULATIONS OF LATE-ACTING LETHAL EFFECTS ON POPULATIONS OF *AEDES* SPP. MOSQUITOES

Mark Q. Benedict[1], Alessandro Bocci[1], Michael A. Robert[2]

[1]University Perugia, Perugia, Italy, [2]University New Mexico, Albuquerque, NM, United States

Mathematical models have been used to predict that late-acting lethal transgenic mosquitoes provide enhanced control of target mosquito populations by maintaining competition in the larval stage. The expected effect is to diminish larval survival among the wild-type larvae, and in comparison with situations in which such competition is not present (e.g. conventional sterile insect technique - SIT), improved control. Mathematical simulations often include simplifying assumptions that might not reflect biological realities and yet provide useful frameworks for predicting the relative value of various control approaches - that is, if they include critical effects of the control measure. While it is usually not possible to simulate large populations with laboratory experiments, it useful to test critical predictions experimentally when such methods can be devised. We will describe experiments conducted to determine whether a previously published model of late-acting lethal transgenic mosquitoes adequately includes critical biological factors of the technology, specifically effects on development rates and survival. We performed laboratory simulations of late-acting lethality and conventional sterile insect technique to determine the effect on the development rate and survival of two *Aedes* species larvae. We also considered the results and novel experimental variables in the context of previous models of control of mosquito populations using late-acting lethals in comparison with SIT.

1886

ANTIMALARIAL AND ANTI-DENGUE PROPERTIES OF A NATURAL *CHROMOBACTERIUM* MOSQUITO MIDGUT COMMENSAL

Raúl G. Saraiva, Yuemei Dong, Abhai Tripathi, José L. Ramirez, George Dimopoulos

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

Plasmodium and dengue virus, the causative agents of the two most devastating vector-borne diseases, malaria and dengue, are transmitted by the *Anopheles gambiae* and *Aedes aegypti* mosquito vectors, respectively. We have identified a novel *Chromobacterium* species in adult *A. aegypti* collected in Panama, *Csp_P*, that can effectively colonize the midgut of *A. gambiae* and *A. aegypti* mosquitoes when introduced through an artificial nectar meal. We have shown that this isolate exerts entomopathogenic activity against both of the mosquito species along with *in vivo* and *in vitro* anti-*Plasmodium* and anti-dengue activities. Interestingly, the well characterized *Chromobacterium violaceum* does not exert such effect. Upon bioassay-guided fractionation of supernatants of a *Csp_P* culture, we were able to map the antiparasitic and antiviral properties to a fraction significantly enriched in a previously characterized cyclic dehydropeptide lactone. This bacterial secondary metabolite was previously pursued as an antifungal, being part of a complex of closely related molecules. We have produced *n*-butanol-based extracts of *Csp_P* cultures that retain *in vitro* activity against blood-stage *Plasmodium* and dengue virus, as well as against the yeast *Saccharomyces cerevisiae*. We are currently pursuing mass spectrometry analysis to characterize the compounds behind the antipathogenic activity of our extracts, along with efforts to identify the gene cluster responsible for production of such compounds by means of both comparative genomics and a transposon-mediated random mutagenesis screening. To our knowledge, this is the first identified bacterium that exerts broad spectrum entomopathogenic and antipathogenic activities, thereby rendering it an interesting candidate for the development of novel vector-borne disease control strategies.

1887

THE EFFICACY OF LONG-LASTING NETS WITH DECLINING PHYSICAL INTEGRITY MAY BE COMPROMISED IN AREAS WITH HIGH LEVELS OF PYRETHROID RESISTANCE

Eric O. Ochomo[1], Nabie M. Bayoh[2], Edward D. Walker[3], Bernard O. Abong'o[2], Maurice O. Ombok[2], Collins Ouma[1], Andrew K. Githeko[4], John M. Vulule[4], Guiyun Yan[5], John E. Gimnig[6]

[1]Maseno University, Kisumu, Kenya, [2]KEMRI/Centers for Disease Control and Prevention, Kisumu, Kenya, [3]Michigan State University, East Lansing, MI, United States, [4]Kenya Medical Research Institute, Kisumu, Kenya, [5]University of California, Irvine, CA, United States, [6]Centers for Disease Control and Prevention, Atlanta, GA, United States

Long-lasting insecticide-treated mosquito nets (LLINs) are a primary malaria prevention tool in sub-Saharan Africa but emergence of insecticide resistance threatens their effectiveness. Cross-sectional surveys of LLINs were conducted in houses of seven and four villages in Gem and Bungoma

Districts in western Kenya, respectively in May 2013. LLIN condition (number and area of holes), number and species of mosquitoes resting inside, and insecticidal activity of LLINs were quantified. Mosquitoes collected inside nets were allowed to lay eggs and the progeny were tested for susceptibility to deltamethrin and permethrin, pyrethoids commonly deployed in LLINs in western Kenya. In Gem, 83.3% of LLINs were less than three years old and 32.4% had at least one hole of any size; while in Bungoma, 92% were less than three years old and 48% had at least one hole. No anopheline and five Culex spp. mosquitoes were found resting inside LLINs in Gem (N=216) regardless of the number and size of holes, while 552 *Anopheles gambiae* s.l., five *An. funestus* s.l. and 137 *Culex* spp. were found inside LLINs (N=216) in Bungoma. The number of mosquitoes resting inside LLINs increased with hole areas >50 cm^2 in Bungoma. In WHO resistance assays, f1 offspring of fed or gravid females collected in nets in Bungoma had 6% and 35% mortaltiy to deltamethrin and permethrin, respectively. LLINs from Bungoma retained strong activity against a susceptible laboratory strain achieving >90% mortality in all bioassays (N=99), but mortality of f1 offspring of field-collected *An. gambiae* s.s. in cone tests was <60% in all assays (N=99) All *An. gambiae* s.s. samples collected in LLINs were homozygous for the kdr genotype L1014S. In conclusion, LLINs develop holes within three years of distribution. In areas with pyrethroid resistance, mosquitoes are able to enter LLINs and survive. LLINs with >50cm^2 of damage were more likely to harbour mosquitoes than nets with no holes. The data indicate that a small amount of damage could compromise the protective efficacy of nets in areas with high levels of pyrethroid resistance.

1888

INDOOR USE OF ATTRACTIVE TOXIC SUGAR BAIT (ATSB) FOR CONTROL OF MOSQUITOES AND FOR RESISTANCE MANAGEMENT

Mark Rowland[1], Zachary Stewart[1], Richard Oxborough[1], Patrick Tungu[2], Matthew Kirby[1], Seth Irish[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]National Institute for Medical Research, Tanga, United Republic of Tanzania

Attractive toxic sugar bait (ATSB), a mixture on insecticide and sugar solution, sprayed onto vegetation has been successful in controlling *Anopheles* mosquitoes outdoors. Indoor application of ATSB has yet to be explored. This study determined whether ATSB stations positioned inside the home would kill host-seeking mosquitoes and constitute a new approach to control of malaria. Classes of insecticides new to malaria vector control were mixed with sugar solution and tested as toxic baits against *Anopheles* in feeding bioassay tests. The most promising ATSB candidates were then trialed in experimental huts in Tanzania against free flying, host seeking mosquitoes. The ATSB stations were hung from ceilings of huts next to untreated mosquito nets occupied by human volunteers. In feeding bioassays, chlorfenapyr (a pyrrole), boric acid and tolfenpyrad (a mitochondrial electron transport inhibitor), mixed in a guava juice-based bait, each killed more than 90% of pyrethroid-susceptible *An. gambiae* s.s. and pyrethroid-resistant An. arabiensis at less than 1% w/v. In the experimental hut trial, the mortality rates of the three ATSB treatments were comparable to long lasting insecticidal nets (LLINs) tested against the same species in the same area. Indoor ATSB constitute a novel application method for insecticide classes that act as stomach poisons and have not been exploited for mosquito control hitherto. Combined with LLIN, indoor use of ATSB has the potential to serve as a strategy for managing insecticide resistance.

1889

FACTORS MEDIATING MATING SUCCESS IN MALE *ANOPHELES GAMBIAE* MOSQUITOES

Adam South[1], Paolo Gabrieli[2], Evdoxia Kakani[1], Flaminia Catteruccia[1]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Università degli Studi di Perugia, Terni, Italy

Human malaria, a major public health burden in tropical and subtropical countries, is transmitted exclusively by female *Anopheles* mosquitoes. Malaria control strategies aimed at inducing sexual sterility in natural vector populations are an attractive alternative due to increasing levels of insecticide resistance. However, the development of these strategies is hampered by a profound lack of knowledge regarding the most basic elements of *Anopheles* mating ecology. Females mate only once, and the suite of mating induced physiological and behavioral changes are predicated in large part upon the transfer of a mating plug containing steroid hormones (SH). Here we report mechanisms of pre and peri-copulatory sexual selection in *An. gambiae* mosquitoes and a role for SH as a possible selective mechanism. High-speed video analysis of mosquito mating swarms revealed definitive evidence of female choice, as females employ specific rejection and acceptance behaviors. Furthermore, video analysis revealed behavioral mechanisms of male competition. We show that successfully mating males are not only larger, but through an ELISA assay we demonstrate that they have significantly higher SH titers in their reproductive accessory glands relative to their unsuccessful counterparts. The mechanisms behind female discrimination is currently being investigated. Additionally, females mated to males with reduced SH levels have lower fecundity and fertility compared to females mated with controls. Given that previous work has demonstrated the importance of male SH in female reproductive phenotypes, fitness in both sexes of *An. gambiae* appears at least partially SH dependent. This work provides critical insights into the mating ecology of a major disease vector and implicates SH as a key factor determining fitness across sequential episodes of sexual selection. Moreover, these results extend our understanding of swarming and monogamous insect mating systems.

1890

STAGE-SPECIFIC, STRUCTURAL PROTEOMES AND THE NODULAR SECRETOME FROM *ONCHOCERCA OCHENGI*, THE CLOSEST RELATIVE OF THE HUMAN RIVER BLINDNESS PARASITE

Stuart D. Armstrong[1], Germanus S. Bah[1], Henrietta F. Ngangyung[2], Dong Xia[1], Samuel Wanji[3], Peter A. Enyong[4], Vincent N. Tanya[2], David W. Taylor[5], Mark L. Blaxter[5], Jonathan M. Wastling[1], **Benjamin L. Makepeace**[1]

[1]University of Liverpool, Liverpool, United Kingdom, [2]Institut de Recherche Agricole pour le Développement, Ngaoundéré, Cameroon, [3]Research Foundation for Tropical Diseases and Environment, Buea, Cameroon, [4]Tropical Medicine Research Station, Kumba, Cameroon, [5]University of Edinburgh, Edinburgh, United Kingdom

The bovine filaria *Onchocerca ochengi* is the closest extant relative of the human river blindness parasite, *Onchocerca volvulus*, and has been used as a natural model of onchocerciasis for two decades. The close parallels between these species include the propensity of the adult worms to form collagenous nodules and their obligate symbiosis with supergroup C strains of *Wolbachia*. To date, in-depth proteomic analysis of filarial structural proteomes has been restricted to a single species, *Brugia malayi,* which has a fundamentally different lifestyle to *O. volvulus.* Here, we report stage-specific proteomes of *O. ochengi* from intrauterine microfilariae, vector-derived L3, and adult female and male worms; alongside host and parasite excretory-secretory products identified in nodule fluid *ex vivo*. We applied a combination of anion exchange fractionation and geLC-MS with interrogation of a draft *O. ochengi* genome assembly to identify >4,600 filarial proteins and 176

proteins from *Wolbachia* strain *w*Oo (33% and 27% of their theoretical proteomes, respectively). Of the filarial proteins, 1,038 (22%) were common to all stages, whereas microfilariae exhibited the greatest number of stage-specific proteins (~920), despite direct harvesting of this material from adult female uteri. Proteins identified by geLC-MS alone accounted for <20% of the total for any single stage, but showed enrichment for membrane transporters, polyubiquitin and respiratory chain components. Preliminary analyses suggested that the relative abundance of galectins, calponins, myosins and antioxidant proteins varied between lifecycle stages. In nodule fluid, >2,000 proteins were identified (77% bovine, 23% filarial, 0.1% bacterial), with strong representation of bovine antimicrobial proteins and filarial transthyretin-like proteins. These data provide a rich resource for comparative analyses of filarial protein expression throughout the lifecycle, as well as supporting research efforts directed at the development of a filarial vaccine, new drugs and diagnostic biomarkers.

1891

TOWARDS IDENTIFICATION AND VALIDATION OF BIOMARKERS FOR THE QUANTIFICATION OF *LOA LOA* MICROFILARIAE (MF) USING PROTEOMIC ANALYSES OF BODY FLUIDS FROM MICROFILAREMIC *LOA*-INFECTED INDIVIDUALS

Papa M. Drame[1], Sasisekhar Bennuru[1], Zaojing Meng[2], Jesica A. Herrick[1], Zhou Ming[2], Thomas B. Nutman[1]

[1]National Institutes of Health, Bethesda, MD, United States, [2]National Institutes of Health, Frederick, MD, United States

Among parasitic helminths, *Loa loa* (Ll) presents a challenge for the mass drug administration programs in areas co-endemic for *Wuchereria bancrofti* and *Onchocerca volvulus* because of the severe adverse events (SAE's) in cases of very high Ll microfilaraemia. To identify microfilarial-derived Ll-specific biomarker(s) that could provide the basis for a microfilarial quantitative immunoassay, we characterized the excretory/secretory (E/S) proteome of Ll mf as well as the Ll-specific proteins found in urine and plasma of Ll-infected and -uninfected individuals using LC MS/MS. From 20 x 10^6 mf purified from the blood of Ll-infected patients and cultured *in vitro,* 1273 proteins (representing 8.2% of the *Ll* putative proteome) were identified. Among the most abundant proteins identified were endochitinase, cyclophilins, and a phosphatidyl ethanolamine binding protein. In addition several hypothetical proteins unique to *Ll* were identified. To further identify if any of these ES proteins were present in body fluids, proteomic analyses of urine and plasma of Ll-infected individuals (depleted of the top 12 to 20 human abundant proteins in plasma) resulted in the identification of 18 (from urine) and 29 (from plasma) Ll proteins found only in Ll-infected individuals that were identified by having at least 2 unique peptides. 4/18 antigens found in urine and 13/20 found in plasma have been selected for biomarker validation based on limited homology to other filarial species and, specific reactivity to polyclonal antibody raised to *Ll* mf ES. In addition, 9 of these tested to date were found to be immunogenic in humans (based on antigen-specific IgG4 reactivity by serum from mf+ Ll-infected plasma (n=30) and not by those from uninfected plasma (n=20)). Development and testing of rapid antigen capture immunoassays are underway to provide an alternative to more standard methods of mf quantification.

1892

VACCINATION WITH *BRUGIA MALAYI*-103 AND *BRUGIA MALAYI*-RAL-2 CONFER SIGNIFICANT PROTECTION AGAINST SUBCUTANEOUS CHALLENGE OF *B. MALAYI* INFECTIVE LARVAE IN MONGOLIAN GERBILS

Sridhar Arumugam[1], Bin Zhan[2], Junfei Wei[2], David Abraham[3], Danielle Ward[1], Sara Lustigman[4], Maria Bottazzi[2], Peter Hotez[2], Thomas Klei[1]

[1]Louisiana State University, Baton Rouge, LA, United States, [2]Baylor College of Medicine, Houston, TX, United States, [3]Thomas Jefferson University, Philadelphia, PA, United States, [4]New York Blood Center, New York, NY, United States

Two *Brugia malayi* proteins, Bm-103 and Bm-RAL-2, are orthologous proteins of confirmed vaccine candidates of *Onchocerca volvulus*. The Ov-103 was identified first as a microfilariae surface associated protein, but later was found to be also expressed in the cuticle, hypodermis and mutivesicular bodies of infective stage larvae of both filarial parasites. Ov-RAL-2 and Bm-RAL-2 are immunodominant proteins expressed in the hypodermis of all stages. Bm-103 was cloned and expressed in *Pichia pastoris* and Bm-RAL-2 was cloned and expressed in Escherichia coli. These recombinant proteins were tested for their efficacy as a vaccine in the *B. malayi* - Mongolian gerbil animal model of lymphatic filariasis. Vaccination was via 3 intraperitoneal injections separated by 2 week intervals. Animals were challenged subcutaneously with 100 infective larvae third stage larvae (L3) and worm recovery was performed 42 or 90 days post infection. Vaccination with Bm-103 administered with alum showed 40% worm reduction in comparison to alum controls. Vaccination with Bm-RAL-2 showed 43% worm reduction in comparison to controls. A fusion protein of Bm-103 and Bm-RAL-2 was created, cloned and expressed in E. coli. Vaccination of gerbils with the Bm-103-Bm-RAL-2 fusion protein induced a 51% worm reduction in comparison to controls. Vaccination of gerbils with the two antigens, Bm-103 and Bm-RAL-2, each injected separately resulted in worm reduction of 69%. The development of embryograms to study the fecundity of female worms harvested from control and vaccinated gerbils are currently underway and will bring insights on impact of vaccination on fertility of female worms. In all vaccination experiments, a strong antigen-specific IgG response was detected by ELISA to the recombinant proteins. Moreover, *in vitro* killing assays using peritoneal exudates cells (PEC) in the presence of gerbil anti-serum against Bm-103 and Bm-RAL-2 showed active killing of L3 larvae in comparison to L3 larvae cultured with appropriate controls sera suggesting that an antibody dependent cell mediated cytotoxicity (ADCC) maybe a potential mechanism of protection. The results suggested that further experiments using these proteins alone or in combination are warranted.

1893

A COMPARATIVE STUDY OF POST-DIETHYLCARBAMAZINE TREATMENT REACTIONS IN ONCHOCERCIASIS AND LOIASIS

Senbagavalli Prakash Babu[1], Jacqueline Herold[1], Fanny Legrand[1], Jesica Herick[1], Eric Ottesen[2], Joseph Kamgno[3], Raceline Gounoue[3], Thomas Nutman[1], Amy D. Klion[1]

[1]National Institutes of Health, Bethesda, MD, United States, [2]The Taskforce for Global Health, Decatur, GA, United States, [3]Center for Filariasis and Other Tropical Disease Research, Yaounde, Cameroon, South Africa

Severe post-treatment reactions occur following diethylcarbamazine (DEC) treatment of filarial infections, including onchocerciasis and loiasis. Although release of the intracellular bacteria, Wolbachia, has been implicated in the pathogenesis of these reactions, *Loa loa* does not contain Wolbachia. The aim of this study was to compare eosinophil, neutrophil and cytokine responses post-DEC treatment in subjects with *L. loa* infection to those in patients infected with *Onchocerca volvulus*. The study included three groups: Group I [LOA-NIH], subjects with microfilaremic (MF+) loiasis treated with DEC (8-10 mg/kg/day for 21 days) at NIH; Group II [LOA-CAM], subjects with MF+ loiasis treated with DEC

(8 mg/kg in a single dose) in Cameroon; Group III [ONCHO], subjects with MF+ onchocerciasis treated with DEC (200 mg/day for 7 days) in Ghana. Complete blood counts and previously collected serum were available at 0h, 4h, 8h, 1-7d and 14d post-initiation of treatment for LOA-CAM and ONCHO and at variable time points for LOA-NIH. The early pattern of eosinophilia post-DEC (a decrease from baseline during the first 24 hours followed by a significant increase over the next 3-5 days) was similar in all 3 groups and the rise was preceded by a transient increase in serum IL-5 levels. In contrast, the % baseline ANC increased significantly post-DEC only in the ONCHO group ($P<0.05$ at days 1,2 and 3 compared to LOA-CAM and LOA-NIH. Serum IL-10 levels increased transiently in all 3 groups, reaching peak values at 1-2 days post-DEC. Although serum TNF-alpha, levels increased at 1-2 days post-DEC in all subjects in the ONCHO group, there was no consistent pattern in the subjects with loiasis. To conclude, parasite antigen release and the resultant Th2-driven eosinophilia may be a major driver of post-DEC reactions in both onchocerciasis and loiasis. The increased TNF-alpha and neutrophilia seen post-DEC in onchocerciasis is likely due to the concomitant release of Wolbachia during microfilarial killing.

1894

ENDOTHELIAL CELLS RELEASE SOLUBLE FACTORS THAT PROLONG THE SURVIVAL OF FILARIAL WORMS *IN VITRO*

Holly Evans, Edward Mitre

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

A major barrier for current mass drug administration (MDA) efforts to control lymphatic filariasis is the inability of current medications to kill adult worms when given as a short course. The development of novel drugs is complicated by the inability to maintain worms for long periods of time *in vitro*, making effective screening of new drugs difficult. In an attempt to improve *in vitro* culture methodology for filarial worms, we have conducted a series of experiments using microfilariae (MF) obtained from gerbils infected with Litomosoides sigmodontis, a filarial parasite of rodents. While the culture of L. sigmodontis MF in Dulbecco's Modified Eagle Medium supplemented with 10% FBS results in an average survival of only 7 days, co-culturing MF with a mouse endothelial cell line (EOMA) expanded survival to 40 days. Not all cell lines have this property, as MF co-cultured with a rat basophilic cell line (RBL-2H3) survived for only 5 days. Culturing EOMA cells in transwell plates extended MF survival to the same degree as direct co-culture, suggesting that the factors microfilariae require are soluble in nature. Heat inactivation of EOMA conditioned media at 56°C reduced MF survival by approximately 50%. However, heat inactivation at 100°C reduced survival to 3 days, signifying that MF require both heat labile and heat stable factors. EOMA cells require FBS to produce these factors, as conditioned media collected from EOMA cells grown in the absence of FBS fail to prolong survival. Importantly, these findings also pertain to adult worms. Both rodent L. sigmodontis and human *Brugia malayi* adult worms also show significantly extended survival when cultured in EOMA conditioned media. We are poised to begin biochemical and comparative analyses to elucidate the chemical nature of these essential factors. Identification of such factors will advance our ability to cultivate filarial pathogens *in vitro* and may provide insights for the development of new anti-filarial compounds.

1895

THE IMPACT OF MATERNAL HELMINTH INFECTIONS ON TH2 RESPONSES AND ATOPIC SENSITIZATION OF INDONESIAN YOUNG CHILDREN

Yenny Djuardi[1], Taniawati Supali[1], Erliyani Sartono[2], Maria Yazdanbakhsh[2]

[1]Department of Parasitology, Faculty of Medicine University of Indonesia, Jakarta, Indonesia, [2]Department of Parasitology, Leiden University Medical Center, Leiden, Netherlands

Immune responses to helminth infection and allergy are both characterized with TH2 responses. Manifestation of allergies in children of low-to-middle income countries were much lower compared to those of affluent countries. We aimed to investigate the impact of maternal helminth infections and other factors on child's development of type 2 responses and atopic sensitization at 4 years of age in an area endemic for filaria and soil-transmitted helminths. Data were collected from pregnant mothers on helminth infections, total IgE and *Ascaris*-specific IgE, education and socioeconomic status (SES). Total IgE and IL-5 in response to mitogen, and helminth antigens were measured in children at 2, 5, 12, 24 and 48 months of age. *Ascaris* and allergen-specific IgE and skin prick testing (SPT) were determined at 4 years of age. Strong TH2 responses were seen at 5 months of age and increased with time. Child's helminth-antigen specific TH2 responses increased significantly with age and were associated with maternal filarial infection, while the increasing of child's general TH2 responses with age were more associated with higher maternal total and *Ascaris*-specific IgE, as well as with low maternal education or SES. Child's *Ascaris*-specific IgE were both associated with child's general and helminth-specific TH2 responses. At 4 years of age when allergen reactivity was assessed by SPT, the high general TH2 responses did not translate into higher SPT. The risk factor for SPT reactivity was low maternal education which decreased the risk of SPT positivity to allergens (adjusted OR, 0.32; 95% CI, 0.12 - 0.87) independently of maternal filarial infection which tended to reduce the child's risk for being SPT positive (adjusted OR, 0.35; 95% CI, 0.07 - 1.70). In conclusion, young children living in areas endemic for helminths developed a strong TH2 responses which was influenced by maternal or child's exposure to helminth infections, but did not translate to a higher SPT reactivity to allergens. This result might explain why the prevalence of allergies in low-to-middle income countries were much lower compared to the more affluent countries.

1896

TRANSCRIPTIONAL PROFILE OF THE *DIROFILARIA IMMITIS* LIFE CYCLE

Ashley N. Luck[1], Christopher C. Evans[2], Molly D. Riggs[2], Laurence Ettwiller[1], Jeremy M. Foster[1], Andrew R. Moorhead[2], Barton E. Slatko[1], Michelle L. Michalski[3]

[1]New England Biolabs, Ipswich, MA, United States, [2]University of Georgia, Athens, GA, United States, [3]University of Wisconsin Oshkosh, Oshkosh, WI, United States

Dirofilaria immitis (Di), or canine heartworm, is a filarial nematode evolutionarily related to those responsible for human parasitic diseases. The *D. immitis* genome, along with the genome of its obligate endosymbiont, *Wolbachia* (*wDi*), was recently completed and published. We initiated a series of transcriptional profiling experiments to better understand the temporal transcriptional activity of *Di* and *wDi* throughout the nematode life cycle. Over 215 million single-end 50 bp reads were generated from total RNA from five *Di* life cycle stages. Based on hierarchical clustering of expression data, nearly 60% of all *Di* genes display stage-specific transcriptional patterns. Pairwise comparison of adult male (AM) and adult female (AF) samples reveals that over 9,000 genes display sex-biased transcriptional patterns. The L3 to L4 transition, which occurs upon entering the mammalian host, is critical to the *Di* life cycle and a potential point of intervention. Among all five life cycle stages examined, a significant portion of *Di* genes are L4-

associated (3525 transcripts), whereas only 65 transcripts show L3-biased expression. Pairwise comparison of the L3 and L4 stages reveals 3157 significantly differentially expressed genes (1170 L3 upregulated, 1987 L4 upregulated) and provides important information regarding transcriptional changes required for this transition. As anticipated, significantly fewer reads mapped to *wDi* genes than to *Di* genes in each life cycle stage. Interestingly, synthesis of the critical metabolite, heme, by *wDi* appears to be synchronized with the production of heme-binding proteins in *Di* in a stage-specific manner. Comparative analysis to human filarial nematodes provides further information on the evolutionary biology of these parasites, while also highlighting opportunities for further drug targeting initiatives. A better understanding of how these genomes function in concert with one another is required for unraveling the complex relationship of the nematode with its endosymbiont, *Wolbachia*, as well as with its canine and mosquito hosts.

1897

EXPERIMENTAL *PLASMODIUM FALCIPARUM* GENETIC CROSSES IN HUMAN LIVER-CHIMERIC MICE

Ashley M. Vaughan[1], Nelly Camargo[1], Matthew Fishbaugher[1], Lisa A. Checkley[2], Richard S. Pinapati[2], Sebastian A. Mikolajczak[1], Michael Ferdig[2], Stefan H. Kappe[1]

[1]Seattle Biomedical Research Institute, Seattle, WA, United States, [2]University of Notre Dame, Notre Dame, IN, United States

Forward genetic studies using experimental genetic crosses are an incredibly powerful tool to pinpoint the genetic basis of phenotypic variation and are typically more powerful than population analysis. For *Plasmodium falciparum* however, only three experimental crosses have been carried out due to the hurdles associated with the process – for ethical reasons humans can not be used and until recently, only splenectomized chimpanzees allowed for the transition from the sporozoite stage to the asexual blood stage of the life cycle necessary for progeny amplification and analysis. Nevertheless, the recombinant progeny from the three crosses led to the discovery of genetic determinants for drug resistance, erythrocyte invasion and blood stage parasite growth. The recent decision by the NIH to cease chimpanzee biomedical research suggests further forward genetic studies are impossible. However, we have shown that a mouse harboring human hepatocytes (the FRG KO huHep mouse) infused with human erythrocytes can support *P. falciparum* sporozoite infection, the completion of liver stage development and the transition to asexual blood stage replication. This thus suggests that *P. falciparum* experimental crosses can be achieved in the FRG KO huHep mouse and here we show here that this is indeed possible and thus this mouse model can replace the previously essential chimpanzee. To achieve our goal, we generated *P. falciparum* gametocytes from the NF54 chloroquine sensitive and GB4 chloroquine resistant parasite lines and used these in mixed feeds to mosquitoes to generate recombinant sporozoite progeny – produced after zygote formation and sexual recombination. Sporozoites were injected into FRG KO huHep mice harboring human erythrocytes and after the liver stage-to-blood stage transition, blood stage parasites were maintained *in vitro*. Parasite cloning and downstream microsatellite analysis revealed the presence of unique recombinant progeny. Furthermore, drug selection demonstrated the creation of recombinant progeny with unique drug resistance patterns not shared by the parental populations. Thus we provide evidence of successful experimental genetic crosses. This methodology should allow for *P. falciparum* "systems genetics" – the study of complex genetic traits in which genomic data and clinical phenotypes are obtained using global "omic" technologies.

1898

MOLECULAR BASIS FOR SIALIC-ACID DEPENDENT RECEPTOR RECOGNITION BY THE *PLASMODIUM FALCIPARUM* INVASION PROTEIN ERYTHROCYTE-BINDING ANTIGEN-140 (EBA-140/BAEBL)

Brian Malpede, Dan Lin, Niraj Tolia

Washington University School of Medicine, St. Louis, MO, United States

Erythrocyte-binding antigen 140 (PfEBA-140/BAEBL) is a *Plasmodium falciparum* erythrocyte invasion ligand that engages Glycophorin C (GPC) on host erythrocytes during malaria infection. PfEBA-140 is a member of the erythrocyte-binding ligand (EBL) family, which contains the four sialic acid dependent invasion proteins utilized by *P. falciparum*. Each of these ligands recognizes a different erythrocyte receptor despite being composed of a highly conserved domain architecture. To elucidate the foundations of receptor specificity within the EBL family and define the structural basis of GPC engagement, we determined two crystal structures of the PfEBA-140 minimal binding domain unbound and in complex with a glycan containing the essential sugar component of GPC that is recognized during erythrocyte engagement. The two domains composing the minimal binding region contain unique structural elements that are likely determinants of receptor specificity. Two glycan binding pockets were observed, one per domain, and the bound sialic acid was modeled into each site. Erythrocyte binding experiments elucidated important glycan contact residues and identified distinct functional roles for the individual sugar binding sites. Our studies provide a structural framework for GPC recognition, form a foundation for future studies of the interaction between PfEBA-140 and erythrocytes, and offer insight into deficient receptor binding and putative receptor switching described for polymorphisms in PfEBA-140. Preventing erythrocyte engagement is an excellent opportunity to inhibit merozoite invasion. Our results will thus aid in the design of rational therapeutics and vaccines that target erythrocyte invasion ligands.

1899

EVOLUTION BEFORE OUR EYES: GENOME MUTATION IN *PLASMODIUM FALCIPARUM*

William L. Hamilton, Antoine Claessens, Mihir Kekre, Thomas D. Otto, Adnan Faizullabhoy, Julian C. Rayner, Dominic Kwiatkowski

Wellcome Trust Sanger Institute, Hinxton, United Kingdom

Genetic mutation is a central process in evolution, and is involved in the emergence of drug resistance and the generation of antigen sequence diversity in the malaria parasite *Plasmodium falciparum*. We studied *P. falciparum* mutation by regularly sub-cloning parasites cultured *in vitro* to isolate single infected red blood cells, so that mutations arising in asexually dividing cells could be detected using whole genome sequencing. We produced the most comprehensive description of *P. falciparum* mutation to date, studying four lab strains, artemisinin resistant Cambodian field isolates and parasites with experimentally induced mutations in DNA repair genes. In total, we analysed >300 genomes from parasites cultured for a total of >1,000 days, capturing hundreds of mutations. We found that: (1) Point mutations are distributed throughout the genome and occur at a similar rate between strains regardless of the drug sensitivity status of the line, contrary to previous studies suggesting that drug resistant parasites are hypermutable; (2) There is a strong mutation bias with G/C to A/T transition mutations over-represented. We estimate this would equilibrate at a similar AT ratio to that observed in the *P. falciparum* genome (~80%); (3) InDels occur predominantly in AT rich low-complexity regions at a higher rate than point mutations, likely due to DNA polymerase slippage events; (4) Structural variation is focused in and around var genes, which encode highly polymorphic PfEMP1 surface-expressed antigens, and this mitotic recombination generates sequence diversity by producing mosaic var genes. With 10^10 parasites in a single infected individual, our data indicate that every nucleotide in the *P. falciparum* genome will undergo point mutations and millions of new mosaic var genes will be produced

every 48-hour life cycle. In summary, we have produced a comprehensive catalogue of *P. falciparum* mitotic genome mutation at all scales from point mutations to interchromosomal translocations, adding considerably to our understanding of parasite genomics and evolution.

1900

IDENTIFYING NOVEL TRAFFICKING COMPONENTS OF THE *PLASMODIUM FALCIPARUM* VIRULENCE FACTOR PFEMP1 THROUGH QTL ANALYSIS

Aaron T. Neal[1], Lisa C. Ranford-Cartwright[2], Richard T. Eastman[1], Chris I. Newbold[3], Rick M. Fairhurst[1]

[1]National Institutes of Health, Rockville, MD, United States, [2]University of Glasgow, Glasgow, United Kingdom, [3]University of Oxford, Oxford, United Kingdom

Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) is the main parasite virulence factor due to its central role in mediating the cytoadherence of infected red blood cells (iRBCs) to the host microvascular endothelium. Directly targeting PfEMP1 as a therapeutic strategy is greatly limited due to the protein's hypervariable nature, which gives rise to approximately 60 different variants. However, interfering with the trafficking of PfEMP1 to the iRBC surface is an attractive approach, as hypervariability becomes inconsequential and previous studies have demonstrated that reduced surface PfEMP1 levels significantly weaken cytoadherence, likely lessening the severity of malaria symptoms and permitting parasite clearance by the spleen. Interestingly, the *in vitro* culture-adapted parasite line 3D7 is inherently defective in exporting PfEMP1 to the iRBC surface. Presuming that PfEMP1 export from the parasite to the iRBC surface is controlled at the genetic level, we hypothesized that 3D7 harbors one or more genetic determinants of impaired PfEMP1 trafficking. To test this possibility, we examined the surface PfEMP1 levels of 17 progeny clones from the genetic cross between 3D7 and the 'trafficking-competent' parasite line HB3. This was assessed using Western blotting and a two-color, triple-layer flow cytometry assay with plasma from malaria-immune Malian adults. Normalized to HB3, we found that 3D7 displays 75% less PfEMP1 on the iRBC surface, with progeny phenotypes ranging from 37% more to 88% less PfEMP1. QTL analysis using 3,597 genome-wide SNP markers identified a significant locus with a LOD score of 4.963 on chromosome 12 that explains approximately 50% of the phenotypic variance. This locus contains a single gene, *Pf3D7_1245600*, encoding a putative kinesin. The role of this gene in the trafficking of PfEMP1 is being confirmed in allele-exchange experiments, where the defect is rescued in 3D7 and introduced in HB3. The results of this study may strengthen our understanding of malaria pathogenesis and provide new targets for much needed therapeutics.

1901

PROTEOMIC COMPOSITION AND SURFACE ACCESSIBLE TARGETS OF *PLASMODIUM* SPOROZOITES

Scott E. Lindner[1], Kristian E. Swearingen[2], Lirong Shi[3], Anke Harupa[4], Ashley M. Vaughan[4], Robert L. Moritz[2], Stefan H. Kappe[4], Photini Sinnis[3]

[1]Pennsylvania State University, University Park, PA, United States, [2]Institute for Systems Biology, Seattle, WA, United States, [3]Johns Hopkins University, Baltimore, MD, United States, [4]Seattle Biomedical Research Institute, Seattle, WA, United States

The transmission of salivary gland sporozoites from an *Anopheline* mosquito initiates the malarial infection of a new mammalian host, which has prompted the development of various therapeutic interventions targeted against sporozoites. While several sporozoite proteins have been historically well studied, systematic proteomic analyses have been hampered by the presence of overwhelming amounts of proteins, nucleic acids, and lipids derived from the dissected mosquito vector. We have overcome this obstacle by developing streamlined purification methods that result in fully infectious sporozoites with very low levels of vector, bacterial, and fungal contamination. These approaches have enabled the most comprehensive total proteomics of two human-infective malaria species (*Plasmodium falciparum* and *P. vivax*), as well as the model rodent-infective species *P. yoelii*. Moreover, these purified sporozoites are also sufficiently devoid of soluble mosquito material to permit the assessment of the surface-accessible proteome of the salivary gland sporozoite. This was done using an amine-reactive crosslinker bearing a cleavable biotin group, which enables high affinity purification and yet leaves a covalent modification of accessible lysines for high confidence identification. We have developed an extensive and stringent washing strategy to minimize the binding of non-specific proteins, which has yielded a greatly expanded surface-accessible proteome above and beyond our previously published list. Several of these candidates have been confirmed with transgenic parasites and the generation of specific antisera. Finally, we have treated purified sporozoites with molecular mimics of mammalian body conditions to observe any differences in protein accessibility or secretion onto the parasite surface in response to these stimuli. Taken together, these characterizations provide a sizeable list of surface accessible proteins that may be valuable new targets for antibody-based interventions.

1902

AGE SPECIFIC INCIDENCE RATES OF MALARIA SUGGEST DIFFERENT RATES OF NATURALLY ACQUIRED IMMUNITY TO MALARIA ACROSS HUMAN HOST GENOTYPES

Oscar A. Nyangiri[1], Meghna Desai[2], Godfrey Bigogo[3], Deron Burton[4], Billy G. Ogwel[3], Allan Audi[3], Joel M. Montgomery[5], Simon Kariuki[3], Thomas N. Williams[6]

[1]Kenya Medical Research Institute-Wellcome Trust Research Programme, Kilifi, Kenya, [2]Malaria Branch, Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Kenya Medical Research Institute-Centers for Disease Control and Prevention, Kisumu, Kenya, [4]National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention, Office of Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, United States, [5]Global Disease Detection Branch, Division of Global Health Protection, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, United States, [6]Imperial College, London, United Kingdom

In endemic areas, the incidence of clinical malaria declines with age, reflecting the role of naturally acquired immunity (NAI) in modulating malaria. Asembo is a malaria endemic area in western Kenya. We investigated whether NAI develops at different rates among children carrying a range of malaria-associated genes by comparing age-specific malaria incidence in children, stratified by genotype. We recruited a birth cohort in March 2012-December 2013 (n=700) from a population under passive surveillance for clinical malaria since August 2006. Additionaly, we recruited birth cohort member siblings <12 years of age into a sibling cohort (n=780) for a combined cohort of 1480 children. Clinical malaria was defined as fever with a malaria-positive blood film in the absence of bacterial co-infections causing fever. Participants were typed for polymorphisms in 40 malaria-associated genes. Age-specific malaria incidence was significantly lower for sickle cell trait (AS) compared to sickle normal (AA) individuals (P<0.001), marginally significant for homozygous(-α/-α) compared to normal ($\alpha\alpha/\alpha\alpha$) alpha thalassemia individuals (P=0.04) and not significant for $\alpha\alpha/\alpha\alpha$ compared to heterozygous(-$\alpha/\alpha\alpha$) individuals . Incidence rates peaked earlier in AA (4-5 years, 0.77 episodes per child per year)compared to AS children (9-10 years, 0.8episodes per child year). For alpha thalassemia, incidence rates peaked earlier in -$\alpha/\alpha\alpha$ (2-3 years, 0.73 episodes per child year), compared to -α/-α individuals (6 years, 1 episode per child per year) and $\alpha\alpha/\alpha\alpha$ individuals (4-5 years, 0.79 episodes per child year). Analyses for the 40 malaria susceptibility genes are ongoing. Preliminary results show shifts in the peaks of age-specific incidence rates by genotype. These findings suggest different rates of NAI among children of different genotypes. Results may be useful in understanding genotype-specific effects of interventions such as malaria vaccines that can modulate NAI.

1903

QUANTIFYING THE INDEPENDENT EFFECTS OF AGE AND EXPOSURE ON TWO COMPONENTS OF MALARIA IMMUNITY: RESTRICTION AND TOLERANCE

Isabel Rodriguez-Barraquer[1], Emmanuel Arinaitwe[2], Prasana Jagannathan[3], Isaac Ssewanyana[2], Agaba Katureebe[2], Moses Kamya[4], Sarah Staedke[5], Grant Dorsey[3], David L. Smith[1], Bryan Greenhouse[3]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Infectious Diseases Research Collaboration, Kampala, Uganda, [3]Department of Medicine, University of California San Francisco, San Francisco, CA, United States, [4]Department of Medicine, Makerere University, Kampala, Uganda, [5]London School of Hygiene & Tropical Medicine, London, United Kingdom

While epidemiologic data consistently suggest that clinical immunity to *Plasmodium falciparum* develops over time in exposed children, lack of accurate measures of exposure and protection have limited our capacity to understand the development of immunity to infection and disease. The objective of this study was to define the acquisition of immunity against *P. falciparum* malaria among children living in endemic areas of varying malaria transmission. In particular, we were interested in measuring the development of clinical tolerance (lack of symptomatic disease given parasitemia, evaluated here by parasite density at which subjects developed objective fever) and parasite restriction (ability to kill or otherwise control the growth of parasites, evaluated here by parasite density). We used data from representative cohort studies being conducted in 100 households from each of three sub-counties in Uganda: Walakuba (aEIR= 3.3), Kihihi (aEIR=31.5) and Nagongera (aEIR=315). The study comprises continuous passive surveillance, active surveillance every 3 months, and monthly mosquito collections in all households. Thus, the dataset used for this analysis included data on over 3400 episodes of clinical malaria and 1400 episodes of asymptomatic parasitemia occurring in 739 children aged 6 months to 11 years of age over two years of follow up. Results from generalized additive models, allowing for flexible interactions between variables of interest, are consistent with strong independent effects of both age and exposure on the development of both tolerance and restriction. Tolerance develops gradually beginning early in life and is not strongly modified by variable exposure. In contrast, restriction starts to develop later in life (4-6 years of age) and depends strongly on cumulative exposure. Further analyses will explore the role of recent and persistent exposure on both of these components of immunity. These findings provide unprecedented insight about the roles of age and exposure on the development of immunity to malaria.

1904

ESTIMATING MALARIA FORCE OF INFECTION ACCOUNTING FOR HETEROGENEITY IN THE RISK OF INFECTION

Levi Mugenyi[1], Steven Abrams[2], Sarah Staedke[3], Niel Hens[2]

[1]Infectious Diseases Research Collaboration, Kampala, Uganda, [2]Centre for Statistics, Interuniversity Institute for Biostatistics and Statistical Bioinformatics, Hasselt University, Diepenbeek, Belgium, [3]London School of Hygiene & Tropical Medicine, London, United Kingdom

The burden of malaria and the intensity of its transmission have been estimated using clinical incidence, parasite rate, and entomological inoculation rate. Less emphasis has been placed on the rate at which individuals become infected, or the force of infection (FOI), the number of new infections per person per unit of time. The above parameters are often estimated without accounting for the intrinsic variability among individuals in the risk of infection. However, such heterogeneity exists due to variability in risk factors, including proximity of residence to mosquito breeding sites, housing structure, density of mosquitoes and frequency of biting, use of prevention measures, differences in surface area between adults and children and in human sweat components, and antimalarial immunity. Here we have proposed approaches for estimating malaria FOI accounting for unobserved heterogeneity using data collected from August 2011 to August 2013 in a cohort of children aged less than 11 years in three sites in Uganda (Tororo, Kanungu and Jinja) with variable malaria transmission intensities. We applied the statistical methodology using linear and nonlinear mixed effects models to estimate both a constant and time-dependent FOI at each site, while accommodating for individual heterogeneity in the acquisition of malaria, and accounting for re-infections. Differences in the FOI were more pronounced between households (variance=2.25) than between children (variance=0.67). The FOI did not vary with time, but differed between the three study sites with higher risk in Tororo (FOI=4.0, 95%CI: 3.4 - 4.6), followed by Kanungu (FOI=0.6, 95%CI: 0.4 - 0.8), and by Jinja (FOI=0.2, 95%CI: 0.1 - 0.3). The FOI was also higher in children above five years of age (FOI=1.5, 95%CI: 1.2 - 1.7), those with symptomatic infection (FOI=0.7, 95%CI: 0.6 - 0.9) and those with anemia (FOI=2.2, 95%CI: 1.7 - 2.7). Therefore, housing structure, individual differences, location of an area, age, symptomatic status and anemia are important factors to consider when estimating the burden of malaria.

1905

SEVERE MALARIAL THROMBOCYTOPENIA: A RISK FACTOR FOR MORTALITY

Daniel A. Lampah[1], Tsin Yeo[2], Michael Malloy[3], Enny Kenangalam[4], Nicholas M. Douglas[2], Paulus Sugiarto[5], Julie A. Simpson[3], Nicholas M. Anstey[2], Nicholas M. Anstey[2], **Ric Price**[2]

[1]Papuan Health and Community Development Foundation, Timika, Indonesia, [2]Menzies School of Health Research, Darwin, Australia, [3]Melbourne School of Population and Global Health, Melbourne, Australia, [4]Mimika District Health Authority, Papua, Indonesia, [5]Rumah Sakit Mitra Masyarakat, Papua, Indonesia

The significance of thrombocytopenia to the morbidity and mortality of malaria is poorly defined. We compared the platelet profiles of patients with and without malaria in southern Papua, Indonesia. Between April 2004 and December 2012 data were available on patient demographics, malaria diagnosis, haematological investigations and clinical outcome in a referral hospital. Of 922,120 patient episodes a total of 215,479 (23.4%) were associated with a platelet measurement, of whom 66,421 (30.8%) had clinical malaria. Patients with *Plasmodium falciparum* monoinfection had the lowest platelet counts with an adjusted odds ratio (AOR) for severe thrombocytopenia (platelet count <50,000 µl-1), compared to those without malaria, of 6.03 [95% Confidence Interval (CI) 5.77-6.30]. The corresponding risks were 5.4 [95% CI 5.02-5.80] for mixed infections, 3.73 [95% CI 3.51-3.97] for *P. vivax* and 2.16 [95% CI 1.78-2.63] for *P. malariae*; p<0.001. In total 1.3% (2,701/215,479) of patients died. Compared to patients with neither severe anemia nor severe thrombocytopenia, those with severe anemia alone had an AOR for death of 5.21 [95%CI 4.53-5.98], those with severe thrombocytopenia alone had an AOR of 4.65 [95%CI 4.10-5.28] and those with both risk factors an AOR of 16.44 [95%CI 13.70-19.74]; p<0.001. In conclusion, severe thrombocytopenia is associated with malarial related mortality. Prospective studies are warranted to define its utility in defining the clinical management of patients with malaria.

1906

EXTENDING THE AGE RANGE FOR SEASONAL MALARIA CHEMOPREVENTION (SMC): EFFECTIVENESS OF SMC IN CHILDREN UNDER 10 YEARS OF AGE DELIVERED THROUGH THE DISTRICT HEALTH SERVICE IN SENEGAL

Elhadji K. Ba[1], B. Cisse[2], C. Pitt[3], J. L. Ndiaye[2], M. Ndiaye[2], J. F. Gomis[2], M. Cairns[3], B. M. Greenwood[3], C. Sokhna[1], O. Gaye[2], P. J. Milligan[3]

[1]Institut de Recherche pour le Developpement, Dakar, Senegal, [2]Universite Cheikh Anta Diop de Dakar, Dakar, Senegal, [3]London School of Hygiene & Tropical Medicine, London, United Kingdom

Seasonal Malaria Chemoprevention (SMC) is recommended for malaria control in children under 5yrs of age where transmission is highly seasonal. In some areas the disease burden may justify extending the age range for SMC. We investigated the effectiveness of SMC in children under 10 years of age in Senegal. SMC with sulfadoxine-pyrimethamine plus amodiaquine was introduced in three districts in a step-wedge design. Fifty-four health posts were randomized to implement SMC starting in 2008, 2009, or 2010, or to remain without the intervention. A surveillance system was established to record all deaths and all malaria cases diagnosed at health facilities, and a pharmacovigilance system was put in place to detect adverse drug reactions. A poisson regression model was used to estimate the effectiveness of SMC in reducing malaria incidence in treated children, with a random effect to account for variation in incidence between health posts. To determine whether SMC was able to reduce malaria transmission, incidence of malaria in age groups too old to receive SMC, was compared between health posts in which SMC was delivered to children, and health posts without SMC, using random-effects poisson regression. SMC was administered to about 14,000 children under 5yrs in 2008, 90,000 children under 10yrs in 2009, and to 155,000 children under 10 yrs in 2010. No serious adverse events attributed to SMC were detected despite a high level of surveillance. Where SMC was delivered, the number of malaria cases in children under 10 years was reduced by 69% (95%CI 65%,72%). Malaria incidence in older age groups was reduced in areas where SMC was delivered to children, by 29% (21%,35%). In conclusion, in some regions of the Sahel and sub Sahel, the age distribution of malaria may justify extending the age range for SMC. Including older children in SMC programmes is safe and effective, and may contribute to reducing transmission.

1907

COMPARISON OF SEASONAL MALARIA CHEMOPREVENTION COVERAGE IN NORTHERN NIGERIA VIA DOOR-TO-DOOR, HEALTH FACILITY AND RETAIL SECTOR DELIVERY

Abigail Ward[1], Justin C. Graves[2], Omowunmi F. Omoniwa[2], Nasir Mohammed Bashir[2], Kathleen Maloney[1], Leslie Emegbuonye[2], Arnaud Le Menach[1], Caitlin F. Dolkart[3], Kabir Masokano[4], Owens Wiwa[2]

[1]Clinton Health Access Initiative, Boston, MA, United States, [2]Clinton Health Access Initiative, Abuja, Nigeria, [3]Clinton Health Access Initiative, Nairobi, Kenya, [4]Kano SMOH, Kano, Nigeria

In 2012, WHO recommended seasonal malaria chemoprevention (SMC) with sulfadoxine-pyrimethamine and amodiaquine (SP-AQ) to prevent malaria in children under five in the Sahel sub-region. As the National Malaria Elimination program in Nigeria plans to apply the recommendation to protect 6 million children in its 9 northern states, identifying the most effective ways to deliver the drug is critical. From August-November 2013, SP-AQ was delivered monthly to under-fives in 3 local government areas of Kano State using 3 delivery mechanisms: door-to-door using community drug distributors, at public health facilities (fixed points), and for a low cost in the private sector. A household survey was conducted in December 2013, one month after the final round of SMC distribution, to assess the coverage achieved through each distribution method. Data were collected on demographics, malaria knowledge, treatment practices, and SMC awareness. We estimated the partial SMC coverage (proportion of under-fives receiving at least one of the four monthly doses) and full coverage (proportion of under fives receiving all doses), and identified factors associated with coverage using multivariable logistic regression models. 176,281 doses of SP-AQ were distributed over four months. The survey collected data from 5,291 children and 3,206 caregivers in 3,079 households. Adjusted partial coverage was significantly higher via door-to-door distribution (86.5%) than via health facility (46.7%) or private sector (27.9%). Full coverage was also highest in the door-to-door delivery arm (56.3%) compared to health facility (19.4%) and private sector (12.2%). Children 1-4 years old were significantly more likely than those <1 year old to receive SMC (p<0.001), and child use of an insecticide-treated bed net was significantly associated with partial coverage (OR=1.4). Door-to-door delivery achieved the highest coverage although a substantial population did not receive SMC. The findings are informing plans for 2014 SMC scale-up across Kano State including community mobilization and sensitization strategies and other fixed-point distribution opportunities.

1908

THE EFFECTIVENESS OF INSECTICIDE TREATED BEDNETS IN HAITI: RESULTS FROM A CASE-CONTROL STUDY

Laura Steinhardt[1], Yvan St. Jean[2], Kimberly Mace[1], Daniel Impoinvil[1], Ryan Wiegand[1], Jean Seme Fils Alexandre[3], Joseph Frederick[2], Roland Oscar[2], Jean Frantz Lemoine[2], Emery Nkuruziza[4], Samuel Jean[4], Brian Wheeler[3], John Barnwell[1], Ellen Dotson[1], Laurence Slutsker[1], S. Patrick Kachur[1], Michelle Chang[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Programme National de la Control de la Malaria, Port-au-Prince, Haiti, [3]Centers for Disease Control and Prevention, Port-au-Prince, Haiti, [4]PSI, Port-au-Prince, Haiti

Insecticide-treated bednets (ITNs) are a cornerstone of malaria prevention, but little evidence exists on their effectiveness in Haiti where the primary vector, *Anopheles albimanus*, has been documented as exophilic and with variable biting times. We conducted a case-control study to assess ITN effectiveness in Haiti, following a national ITN distribution campaign in 2012. Patients presenting to outpatient departments were systematically screened for fever or history of fever. Eligible patients were administered a brief questionnaire and blood was collected for a malaria rapid diagnostic test (RDT) and dried blood spots. From September 2012-February 2014, 9,318 patients, including 379 (4.1%) RDT-positive patients, were enrolled across 17 health facilities in five departments in Haiti. Retrospectively matching up to four RDT-negative controls per RDT-positive case by age group, sex, location of residence, and enrollment period yielded 365 cases and 1,204 RDT-negative controls. Slightly more than half (57.1%) of patients reported owning any bednet, with no difference among matched cases and controls. We found no difference in the proportion of cases and controls who reported using any bednet (34.5% vs. 32.9%, p=0.39) or a campaign ITN (21.9% vs. 19.5%, p=0.30) the previous night, or always using a campaign ITN in the two weeks before their illness (18.4% vs. 18.5%, p=0.84). In a multivariabe conditional logistic regression model, consistent use of a campaign ITN was not related to RDT positivity. The only variable related to RDT positivity in the model included body temperature (Odds Ratio = 1.40, 95% Confidence Interval: 1.25, 1.57 per one-degree Celsius increase). Additional entomologic investigation found that all *Anopheles* mosquitoes tested from the study areas were susceptible to permethrin, the insecticide used on campaign ITNs. Our results based on RDT status do not provide evidence to support ITNs as an effective malaria prevention strategy in Haiti. Additional results using a PCR-based case definition will be presented.

1909

AEDES AEGYPTI FEMALE MOSQUITOES WITH ALANINE AMINOTRANSFERASE DEFICIENCY FACE A STRESSFUL METABOLIC CHALLENGE DURING BLOOD DIGESTION

Jun Isoe[1], Stacy Mazzalupo[1], Virginia Belloni[2], **Patricia Y. Scaraffia**[2]

[1]The University of Arizona, Tucson, AZ, United States, [2]Tulane University, New Orleans, LA, United States

We have recently evaluated the exposure of *A. aegypti* females to L-cycloserine (LCS), a well-known inhibitor of alanine aminotransferase (ALAT) in animals. Our results indicated that 10 mM LCS interferes with *Aedes aegypti* blood metabolism causing motor impairment and a 35% of mortality with an acute effect during the first 6 hours after treatment. Interestingly, only 11% of the total mortality was observed between 24 and 72 hours after feeding. In order to follow up this finding, the expression pattern of two genes encoding ALAT (1 and 2) was first analyzed in sucrose- and blood-fed *A. aegypti* tissues by qRT-PCR. ALAT1 and ALAT2 transcript levels exhibited a distinct expression pattern in mosquito tissues dissected during a gonotrophic cycle. Next, RNAi-mediated gene silencing was used to knock down endogenous levels of each transcript in mosquito tissues. Injection of female mosquitoes with either dsRNA-ALAT1 or dsRNA-ALAT2 or both (dsRNA-ALAT1/2) significantly decreased the expression of ALAT1 or ALAT2 or ALAT1/2 in fat body (FB) and Malphigian tubules (MT) at 24 hours after blood feeding, when compared to dsRNA-firefly luciferase-injected control. As expected, the expression of ALAT1 was not modified in tissues from dsRNA-ALAT2-injected females and vice versa. Western blot analysis demonstrated that the protein levels of ALAT were also significantly reduced in tissues of dsRNA-ALAT-injected females when compared to control mosquitoes. Moreover, the knockdown of *A. aegypti* ALAT1 or ALAT2 or ALAT1/2 caused unexpected phenotypes such as a delay in blood digestion, a massive accumulation of uric acid in the midgut posterior region, and a significant decrease in nitrogen waste excretion during the first 48 hours after blood feeding. Concomitant with these results, the expression of genes encoding both the ammonia transporter and xanthine dehydrogenase were significantly increased in FB and MT of dsRNA-ALAT-injected females. These findings highlight the efficient and complex mechanisms that blood-fed mosquitoes use to avoid ammonia and free radical toxicity.

1910

ANOPHELES GAMBIAE SMALL-RNA PATHWAYS IMMUNITY TO DIFFERENT PATHOGENS

Guillaume Carissimo[1], Emilie Pondeville[1], Emmanuel Bischoff[1], Catherine Bourgouin[1], Anna-Bella Failloux[1], Christophe Antoniewski[2], Alain Kohl[3], Kenneth D. Vernick[1]

[1]Institut Pasteur, Paris, France, [2]Université Pierre et Marie Curie - UPMC, Paris, France, [3]University of Glasgow - MRC Center for Virus Research, Glasgow, United Kingdom

Many mosquito species are vectors of human pathogens, such as viruses responsible for Dengue or Chikungunya, or malaria parasites. Mosquitoes from the *Aedes* genus mainly transmit viruses, whereas the *Anopheles* genus is exclusively responsible for transmission of human malaria. The basis for these pathogen-vector specificities is currently unknown. Mosquito small RNA pathways have many functions such as regulation of genes in the development or immunity. Previous studies in *An. gambiae* have shown that (i) regulation of anti-*Plasmodium* immunity by microRNA was essential for correct mosquito protection, and that (ii) the siRNA pathway is essential for the control of arboviral infection after intrathoracic inoculation. Using next generation sequencing and functional genomics to examine *Anopheles gambiae* mosquitoes infected with O'Nyong Nyong virus and *Plasmodium* parasites, we were able to (i) discover new *Anopheles* microRNAs and characterize specific microRNAs that are regulated upon infection; (ii) show that the siRNA pathway does not contribute to antiviral defense during early arboviral infection in the midgut while it is protective at later stages in the systemic compartment; (iii) and implicate the siRNA pathway in evasion of immunity by malaria parasites in the midgut. These results expand our understanding of the small RNA immunity machinery in an important African vector.

1911

STRUCTURAL DIVERGENCE OF HETEROCHROMATIN BETWEEN INCIPIENT SPECIES OF *ANOPHELES GAMBIAE*

Atashi Sharma, Maria V. Sharakhova, Igor V. Sharakhov

University of Virginia Tech, Blacksburg, VA, United States

The major African malaria vector *Anopheles gambiae* is known to be undergoing incipient speciation into two molecular forms – M and S – recently named *An. coluzzi* and *An. gambiae*, respectively. Heterochromatin plays a vital role in several important biological functions, and is known to be associated with longevity and individual fitness of organisms as well as with postmating reproductive isolation between species. The genome of *Anopheles gambiae* was first sequenced in 2002 and has been updated since but still contains important information gaps with regards to the repetitive DNA content in heterochromatin. In order to achieve a better understanding of differentiation within *An. gambiae*, it is essential to develop a physical map containing information about the repetitive DNA and determine the differences in heterochromatin between the incipient species. Using multiple strains of M and S forms, we mapped repetitive DNA sequences including satellite DNA and ribosomal DNA (rDNA) with respect to bands of pericentric heterochromatin of mitotic chromosomes. Satellite DNA probe Ag53A hybridized to the pericentric heterochromatin/rDNA locus junction in both forms. However, unexpectedly, satellite DNA AgY53B hybridized at the base of proximal band in the M form but at the tip of the band in the S form, indicating a possible shift or inversion in the satellite DNA position during divergence of these forms. Satellite DNA AgY477 depicted a similar pattern, hybridizing to different positions between the forms. Idiograms based on above information were prepared for the M and S forms as well as other members of the *An. gambiae* complex, serving as a tool in better understanding of evolution of the repeat rich regions in the *An. gambiae* genome. Our results revealed that the rapid evolution of heterochromatin is not restricted to species with the postmating reproductive isolation. The structural reorganization of satellite DNA observed between the M and S forms suggests a possible role of heterochromatin in initial diversification of malarial vectors.

1912

X-CHROMOSOME LOCALIZED RECOMBINATION HOTSPOTS UNDERMINE EXISTING MOLECULAR DIAGNOSIS OF *ANOPHELES GAMBIAE* AND *AN. COLUZZII* UNDER HIGH HYBRIDIZATION

Beniamino Caputo[1], Verena Pichler[1], Emiliano Mancini[1], Marco Pombi[1], Josè Vicente[2], Amabelia Rodrigues[3], Joao Dinis[3], Vincenzo Petrarca[4], Joao Pinto[5], Alessandra della Torre[1], David Weetman[6]

[1]Department of Public Health and Infectious Diseases, Università di Roma "La Sapienza", Italy, Rome, Italy, [2]Centro de Malária e outras Doenças Tropicais, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisboa, Portugal, [3]Instituto Nacional de Saúde Pública, Ministério da Saúde Pública, Bissau, Guinea-Bissau, [4]Department of Biology and Biotechnology, Università di Roma Sapienza, Rome, Italy, [5]Centro de Malária e outras Doenças Tropicais, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisboa, Portugal, [6]Vector Group, Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Anopheles gambiae and *An. coluzzii* are defined based on SNPs in the multi-copy IGS chromosome-X linked rDNA region. In most of their range of sympatry, where they rarely hybridize, multiple areas of the genome are differentiated, with highest divergence found toward centromeres. However, genomic divergence varies markedly with levels of introgression.

In a putative secondary contact region in Guinea Bissau, where hybrid females have been observed at frequencies >20%, differentiation is largely limited to a region proximal to the X-centromere. This region may play a critical role in the speciation process, but the evolutionary forces acting upon it are poorly understood. The aim of our work was to evaluate inter-specific genomic differentiation and frequency of recombination along the X-centromere in the Guinean hybridization region, focusing the analysis on X-chromosome hemizygous males to permit unambiguous haplotype analysis. We genotyped 263 males for the IGS diagnostic locus and for two additional markers about 1 Mb from it: i) the insertion of a SINE retrotransposable element specific for *An. coluzzii*, which is widely used as a species diagnostic; and ii) a 57 bp-insertion in intron 4 of cytochrome CYP4G16 (CYP) gene specific for *An. gambiae*. Moreover, using Illumina and Sequenom genotyping we characterised almost 800 SNPs (34 of which are species-specific and located in the X centromeric region) in 59 males. We observed: i) lack of inter-specific differentiation in the overall genome, with the exception of chromosome-X centromere; ii) intra-individual mixed IGS-arrays in 12% of the whole male sample, suggesting the occurrence of introgression events; iii) unexpected recombination among IGS, SINE and CYP in 24% of the males, and between SINE and CYP in 13% of them despite the close proximity of these 2 loci (7 Kb). Moreover, results from SNP-genotyping showed: i) some, although low, levels of recombination in the X-centromere; ii) introgression in the IGS-region in the absence of recombination in the X-centromere; iii) a hot-spot of recombination nearby the SINE-insertion. The results highlight (1) the likely importance of reduced recombination in maintaining integrity of the X-genomic island of divergence with high gene flow, consistent with genetic-hitchhiking-based speciation models and (2) the poor reliability of existing diagnostics (IGS, SINE) for the two species in the secondary contact region.

1913

AN INTEGRATED CHROMOSOME, GENETIC LINKAGE AND GENOME MAP FOR THE SOUTHERN HOUSE MOSQUITO *CULEX QUINQUEFASCIATUS*

Anastasia N. Naumenko[1], Vladimir A. Timoshevskiy[1], Becky S. deBruyn[2], David W. Severson[2], Igor V. Sharakhov[1], Maria V. Sharakhova[1]

[1]Fralin Biotechnology Center, Virginia Tech, Blacksburg, VA, United States, [2]Department of Biological Sciences, Eck Institute for Global Health, University of Notre Dame, Notre Dame, IN, United States

Culex quinquefasciatus, a southern house mosquito, is a member of geographically widespread mosquito complex species with high variation in behavioral patterns and ability to transmit diseases, including lymphatic filariasis and West Nile fever. Only 10.4 % of the 579 Mb genome is currently assigned to the chromosomes based on genetic linkage mapping. Although cytogenetic maps for the polytene chromosomes for this mosquito were developed, their utilization for the genome mapping remains difficult because of the low number of high quality spreads in chromosome preparations. We constructed idiograms for mitotic chromosomes of *Cx. quinquefasciatus* based on their banding patterns at early metaphase. These idiograms represent the first cytogenetic map developed for mitotic chromosomes of *Cx. quinquefasciatus*. Genetic contigs associated with 14 major genetic markers, 18S rDNA and 10 largest contigs were anchored to the exact positions on *Cx. quinquefasciatus* chromosomes using fluorescent *in situ* hybridization. The order of genetic markers was consistent with the previously developed genetic linkage map. Some new insights were provided into chromosome evolution in mosquitoes. For example, FISH result of 18S rDNA suggests an inverted position of the ribosomal locus in chromosome 1 of *Cx. quinquefasciatus* compared with Ae. aegypti. This locus was mapped close to the centromere above the heterochromatin band in *Cx. quinquefasciatus* but in the middle of the 1q arm below the heterochromatin band in Ae. aegypti. Our study in progress linked chromosome and genetic linkage maps with 4.8% of the *Cx. quinquefasciatus* genome.

1914

MICROSATELLITE AND DNA SEQUENCE POPULATION GENETICS EVALUATION OF THE SOUTHWEST PACIFIC MALARIA VECTOR *ANOPHELES KOLIENSIS* (OWEN)

Nigel W. Beebe[1], Robert D. Cooper[2], Weixin Xu[3], Sarah L. Fordyce[4], Luke Ambrose[3]

[1]School of Biological Sciences, University of Queensland and CSIRO, Brisbane, Australia, [2]Australian Army Malaria Institute, Brisbane, Australia, [3]School of Biological Sciences, University of Queensland, Brisbane, Australia, [4]Department of Forensic Medicine, University of Copenhagen, Copenhagen, Denmark

The southwest Pacific malaria vector *Anopheles koliensis* Owen is one of 13 cryptic members of the *Anopheles punctulatus* group that can only be identified by molecular tools. Its distribution in Papua New Guinea (PNG) has only recently been described and it is found predominantly throughout inland lowlands and river valley flood plains below 300m and is common throughout the continual wet Sepik, Ramu, and Markham River valleys on the north side of PNG's central ranges as well as throughout the northern and southern lowland region of PNG's Papuan Peninsula. *An. koliensis* utilizes both natural larval habitat (ground pools and swamps), as well as human modified habitat (vehicle wheel tracks and drains). In this study, we drill into the population genetic of *An. koliensis* in PNG - a region of incredible biogeography - to detail the spatial and genetic connectivity of this malaria vector species. We evaluate nuclear and mitochondrial DNA sequence as well as develop and analyse 12 microsatellites. We find a species with overt genetic and geographic population structure that can be explained, in most cases, by natural barriers. We do not find evidence to support the existence of intraspecific rDNA genotypes previously described but we do find *An. koliensis* to be a single species with a long history in New Guinea.

1915

CHARACTERIZATION OF MALE REPRODUCTIVE FACTORS ESSENTIAL FOR MATING SUCCESS IN THE LIFE CYCLE OF *ANOPHELES GAMBIAE* MOSQUITOES

Evdoxia Kakani[1], Sara Mitchell[2], Paolo Gabrieli[1], Enzo Mameli[1], Ainhoa Mariezcurrena[3], Flaminia Catteruccia[2]

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

Mating is a vulnerable step in the lifecycle of *Anopheles gambiae* mosquitoes, as females of this species mate only once. Seminal fluids produced in the male accessory glands (MAGs) and transferred during mating are likely to induce permanent refractoriness to further insemination in females. Moreover, these factors are likely to play a key role in other post-mating processes, including egg laying and fertility. However, the identity of these male molecular triggers is largely unknown. As changes in levels of individual semen components after mating may indicate factors crucial for mating success, we performed a time course transcriptional analysis of MAG genes at 3 time points (3h, 12h, 24h) after mating, representing the period between 2 mating events in the field. Mated tissues were compared to those from virgin males in 4 replicates using whole-genome microarrays. Surprisingly, a total of 4,319 genes were differentially expressed after mating ($p<0.05$ FDR). Gene enrichment analysis revealed a number of functional groups significantly enriched in the dataset. During early time points after mating, genes associated with RNA transcription, translation and post-translational modifications were enriched, suggesting an induction of pathways essential for the replenishment of MAG content. At the latest time point enrichment was observed in genes involved in protein export, indicative of males preparing for the next mating event. Interestingly, genes involved in hormone biosynthesis were also highly enriched, supporting previous findings that male hormones may play a critical role in *An. gambiae* reproduction. We then investigated the function of male hormones that are replenished after mating. Tampering with the synthesis of these hormones in males

had dramatic effects on the reproductive physiology of mated females, which showed strongly reduced fecundity and fertility. These results reveal previously unknown pathways that are key to mosquito reproductive physiology, and offer novel targets for vector control efforts aimed at reducing mosquito reproductive success.

1916

AN INPUT ON PREVENTIVE STRATEGIES FROM THE FIELD: IRON LEVELS AND INTERMITTENT PREVENTIVE TREATMENT (IPTP) CALENDAR ARE ASSOCIATED WITH *PLASMODIUM FALCIPARUM* PARASITEMIA DURING THE FIRST YEAR OF LIFE

Violeta Moya-Alvarez[1], Smaila Ouedraogo[2], Florence Bodeau-Livinec[3], Gilles Cottrell[2], Michel Cot[4]

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

Malaria is the disease with the highest infant mortality and morbidity worldwide. In 2012 the WHO reported over 207 million cases and more than 627,000 deaths. In Benin malaria is the leading cause of mortality (23%) among children under 5 years. There are significant differences in *P. falciparum* parasitemia among children during the first year of life. We aim at describing the factors contributing to malaria episodes and to high parasitemia during the first year of life in Benin. Therefore we have investigated the association of maternal exposure to the vector *in utero* (determined by pregnancy associated malaria (PAM) and the intermittent preventive treatment (ITPp)) to infant parasitemia analyzing as well nutritional, environmental and socio-economic risk factors. 1000 pregnant women and 400 of their children were followed during pregnancy and the first year of life in Allada (Benin), between 2010 and 2012. At inclusion socio-demographic status and gyneco-obstetric history were investigated. Extensive medical and biological exams were realized with both doses of IPTp and at delivery for the mothers and at 6, 9, and 12 months for the infants. Further exams were realized at each emergency consultation. All patients were treated in case of disease. Random coefficient models assessed the relationship between the different parasitemia measures in infants and other variables. A novel approach consisting in pathway analysis was used to analyze the evolution pattern of parasitemia. Maternal age at both IPTp doses, infant weight, mother parasitemia at delivery, number of emergency consultations and total body iron were correlated with infant parasitemia. Placental malaria was not correlated with infant parasitemia when adjusting for mother parasitemia at delivery. We find for the first time that IPTp has not only an effect on LBW but also on infant parasitemia. Therefore IPTp calendar should cover extensively the pregnancy and protect both the mother and the infant. Total body iron is also correlated with infant parasitemia. WHO recommends supplements with iron and folic acid when the prevalence of anaemia exceeds 40%. However the Pemba study and a Cochrane review conclude to an increased risk for malaria among supplemented children in the context of limited malaria coverage. Our results confirm the association between iron and malaria and plead for protective measures in the context of iron supplementation

1917

MALARIA-TRANSMISSION INTENSITY AND THE PROTECTIVE EFFECT OF INTERMITTENT PREVENTIVE TREATMENT (IPTP): POLICY IMPLICATIONS FOR THE ANTENATAL CARE OF PREGNANT WOMEN IN SUB-SAHARAN AFRICA

R. Matthew Chico, Jorge Cano, Cono Ariti, Timothy Collier, Daniel Chandramohan, Brian Greenwood

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

The World Health Organization (WHO) recommends the provision of intermittent preventive treatment with sulphadoxine-pyrimethamine (IPTp-SP) to pregnant women resident in areas of moderate (stable) or high malarial transmission to prevent low birthweight (LBW), neontal deaths and maternal anemia. The incidence of malaria below which IPTp-SP no longer provides a cost-benefit is unknown, but it is important to estimate as countries make progress towards malaria elimination. We conducted a review of IPTp-SP studies and matched the protective efficacy of IPTp-SP against LBW to a proxy measure of malarial incidence in women of the same studies, the prevalence of malaria in children. The latter was calculated in two ways. We first extracted prevalence estimates in children from the Malaria Atlas Project (MAP) database and, for the second measure, we selected and then pooled the point prevalence data that underly the MAP estimates using random-effects models. We then applied meta-regression models of the protective efficacy of IPTp-SP against LBW to the estimates of malarial prevalence in children calculated in both ways, and stratified results by gravidity. Among multigravidae, the protective effect of IPTp-SP against LBW was no longer significant in areas where the malarial prevalence in children was < to 9% when we applied MAP estimates, and < to 8% using our pooled estimates. The latter analysis showed a significant linear trend (P=0.043). Malarial transmission intensity could not explain variations in the efficacy of IPTp-SP among paucigravidae. IPTp-SP no longer protects against the incidence of LBW among multigravidae in geographical areas of 20 sub-Saharan countries where the parasite prevalence among children is < 8%. In contrast, our analysis among paucigravidae suggests that two or more doses of SP is protective against LBW in transmission settings that are below the current recommendation set by the WHO.

1918

MISSED OPPORTUNITIES FOR DELIVERING PREVENTIVE TREATMENT FOR MALARIA IN PREGNANCY DURING ANTENATAL CARE AND COMPARISON WITH DELIVERY OF NEONATAL TETANUS PREVENTION: AN ANALYSIS OF HOUSEHOLD SURVEYS IN SUB-SAHARAN AFRICA

Kathryn Andrews[1], Michael Lynch[2], Erin Eckert[3], **Julie Gutman**[2]

[1]Harvard School of Public Health, Cambridge, MA, United States, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]President's Malaria Initiative, United States Agency for International Development, Washington, DC, United States

Coverage of intermittent preventive treatment for malaria during pregnancy (IPTp), a potentially lifesaving intervention, remains low in Africa, despite high antenatal care attendance among pregnant women and recommendations for IPTp to be given at each antenatal care visit after the first trimester. To highlight areas of potential improvement, we assessed trends in IPTp coverage over time, and identified the missed opportunities to deliver IPTp at antenatal clinics. Data from 66 national household surveys conducted in 31 sub-Saharan African countries between 2000 and 2012, with relevant questions on antenatal care (ANC) were used to determine the coverage of ANC visits, IPTp and tetanus toxoid (TT). Missed opportunities for IPTp were calculated by comparing the number of IPTp doses received to the number of ANC visits during which IPTp could be given. To account for visits occurring in the first trimester when IPTp is not given, one visit was subtracted from the total number of visits for women who reported having their first visit in the first trimester. The median proportion of pregnant women receiving at least 2 doses of IPTp was 1.0% (IQR 0-10.3%) during 2000-2007 and 27.2% (IQR 13.9-42.1%) during 2008-2012. Missed opportunities for IPTp delivery occurred in a median 99.2% (IQR 90.2-100%) of ANC visits from 2000-2007 and 76.8% (IQR 65.6-92.9%) from 2008-2012. The median proportion of primigravid women receiving at least 2 doses of TT is much higher: 50.2% (IQR 34.4-64.0%) during 2000-2007, and 59.2% (IQR 48.6-64.9%) during 2008-2012. With the exception of two countries, the proportion of primigravid women receiving at least 2 doses of IPTp is lower than the proportion receiving at least 2 of TT: the median absolute difference is 41.7% from 2000-2007 (IQR 29.2-54.1%) and 36.7% (IQR 23.7-40.6%) from 2008-2012. Although IPTp coverage has increased slightly over time, levels remain disappointingly low, and missed opportunities for IPTp occur at the majority of ANC visits. Although

both are delivered through the ANC, delivery of IPTp occurs much less frequently than delivery of TT, suggesting that barriers to IPTp delivery could be overcome. Further work is required to determine the specific factors that are driving the surprising discrepancies between IPTp and TT coverage, with an eye toward improving IPTp coverage through potential linkage with the TT administration infrastructure.

1919

PHARMACOKINETICS OF ARTEMETHER-LUMEFANTRINE IN PREGNANT AND NON-PREGNANT WOMEN WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN WESTERN KENYA

Elizabeth A. Juma[1], Bernhards R. Ogutu[1], Florence Oloo[2], Tara Barwa[2], Rashid Aman[3]

[1]Kenya Medical Research Institute, Nairobi, Kenya, [2]Centre for Research in Therapeutic Sciences, Nairobi, Kenya, [3]African Centre for Clinical Trials, Nairobi, Kenya

Pregnancy increases the vulnerability to malaria infection in all women living in areas of malaria risk. The World Health Organization recommends the use of artemisinin-based combination therapies (ACTs) for treatment of acute uncomplicated falciparum malaria in the second and third trimesters of pregnancy. The pharmacokinetic properties of antimalarial drugs are often affected by pregnancy, resulting in lower drug concentrations and a consequently higher risk of treatment failure. While artemether-lumefantrine is used in Kenya and most Eastern Africa countries as first line treatment for malaria in pregnancy in the second and third trimesters, its pharmacokinetics in pregnant African women with malaria is not well characterized. This study evaluated the population pharmacokinetics of artemether, dihydroartemisinin, lumefantrine and desbutyl-lumefantrine in 45 pregnant and 25 non-pregnant women with uncomplicated malaria in Western Kenya. All patients were treated with the standard fixed dose artemether-lumefantrine 20/120mg tablets over 3 days. Frequent venous blood sampling was obtained over the treatment period for pharmacokinetic evaluation. Estimates for pharmacokinetic and variability parameters will be obtained through nonlinear mixed effects modeling. Simultaneous modeling of parent drug and metabolite will be used for both artemether and lumefantrine. Absorption and clearance of artemether-lumefantrine in pregnant compared with non-pregnant African women with uncomplicated malaria and the implications of findings will be presented.

1920

A STUDY OF THE PHARMACOKINETICS OF PRIMAQUINE IN LACTATING WOMEN AND BREASTFED INFANTS FOR THE RADICAL TREATMENT OF UNCOMPLICATED MATERNAL *PLASMODIUM VIVAX*

Mary Ellen T. Gilder[1], Joel Tarning[2], Niklas Lindegårdh (posthumous)[2], Germana Bancone[1], Warunee Hanpithakphong[2], Hilda Moo[1], Tun Tun Win[1], Nicholas White[3], François Nosten[3], Rose McGready[3]

[1]Shoklo Malaria Research Unit, Mae Sot, Tak, Thailand, [2]Mahidol Oxford Research Unit, Bangkok, Thailand, [3]Centre for Tropical Medicine, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom

Women of reproductive age in malarial areas of the world suffer due to lack of evidence on the safety of effective antimalarials in pregnancy and lactation. *Plasmodium vivax* recurrences are more common during pregnancy but the only widely available medication for radical treatment of *P.vivax*, primaquine, is contraindicated in pregnancy. The postpartum period presents a key opportunity for radical treatment of *P. vivax*, but there are no studies quantifying primaquine excretion in breast milk and the dose that breastfed infants would be exposed to is unknown. We are conducting the first-ever study of the pharmacokinetics of primaquine lactating women and their breastfed infants during a 14-day radical treatment of *P.vivax*. Twenty-four healthy lactating women at risk for recurrent malaria (i.e. with a history of *P. vivax*) and their infants (at least 28 days old) are being recruited for detailed pharmacokinetic study. Prior to enrolment, G6PD deficiency is excluded by rapid qualitative fluorescent spot test and G6PD genotype from PCR spot. Anemic patients are treated and enrolment is delayed until normal HCT is established. Hemoglobin typing is analyzed and fetal hemoglobin in infant blood is quantified. Primaquine is administered to eligible mothers at a dose of 0.5 mg/kg/day and is directly observed. Primaquine and carboxyprimaquine levels are measured by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) on venous and capillary plasma, urine, saliva and breast milk samples from the mothers, as well as capillary plasma samples from the infants. There have been no drug-related adverse events to infants, though several women have experienced mild to moderate methemaglobinemia (not requiring treatment). Preliminary data shows low but measurable levels of primaquine in both breast milk and infant plasma. The final results of this study could have profound impacts on malaria control and women's health in the tropics.

1921

SAFETY OF ARTEMETHER-LUMEFANTRINE EXPOSURE IN EARLY PREGNANCY: AN OBSERVATIONAL COHORT

Dominic Franklin Mosha

Ifakara Health Institute, Dar es salaam, United Republic of Tanzania

There is limited data available regarding safety profile of artemisinins in early pregnancy. They are therefore not recommended by WHO as a first line treatment for malaria in first trimester due to associated embryo-foetal toxicity in animal studies. The aim of the study was to assess birth outcome among pregnant women inadvertently exposed to AL during first trimester in comparison to those of women exposed to other antimalarial drugs or no drug at all during the same period of pregnancy. Pregnant women with gestational age < 20 weeks were recruited from Reproductive and Child Health (RCH) clinic or from monthly house visits (demography surveillance), and followed prospectively until delivery. A structured questionnaire was used to interview participants. 2167 pregnant women were recruited and 1783 (82.3%) completed the study until delivery. 319 (17.9%) used antimalarials in first trimester, of whom 172 (53.9%) used artemether-lumefantrine (AL), 78 (24.4%) quinine, 66 (20.7%) sulfadoxine-pyrimethamine (SP) and 11 (3.4%) amodiaquine. Quinine exposure in first trimester was associated with an increased risk of miscarriage/stillbirth (OR 2.5; 1.3 - 5.1) and premature birth (OR 2.6; 1.3 - 5.3) as opposed to AL with (OR 1.4; 0.8 - 2.5) for miscarriage/stillbirth and (OR 0.9; 0.5 - 1.8) for preterm birth. Congenital anomalies were identified in 4 exposed groups namely AL only (1/164 [0.6%]), quinine only (1/70 [1.4%]), SP (2/66 [3.0%]), and non-antimalarial exposed group (19/1464 [1.3%]). Exposure to AL in first trimester was more common than to any other antimalarial drugs. Quinine exposure was associated with adverse pregnancy outcome, which was not the case following other antimalarial intake. Since AL and quinine were used according to their availability rather than to disease severity, it is likely that the effect observed was related to the drug, and not to the disease itself. Detailed information on developmental milestone up to 12 months is ongoing to rule out any adverse effect on infancy as a result of AL exposure in first trimester. Even with this caveat, a change of policy from quinine to AL for the treatment of uncomplicated malaria during the whole pregnancy period could be already envisaged.

1922

SAFETY AND EFFICACY OF FOUR ARTEMISININ-BASED COMBINATION TREATMENTS IN AFRICAN PREGNANT WOMEN WITH MALARIA

Umberto D'Alessandro[1], Halidou Tinto[2], Linda Kalilani Phiri[3], Michael Nambozi[4], Harry Tagbor[5], Maminata Traore[2], Innocent Valea[2], Victor Mwapasa[3], Gertrude Kalanda[2], Modest Mulenga[4], Joris Menten[6], Raffaella Ravinetto[6], Yves Claeys[6], Maaike De Crop[6], Theonest Mutabingwa[7]

[1]Medical Research Council, Fajara, Gambia, [2]Clinical Research Unit, Nanoro, Burkina Faso, [3]College of Medicine, University of Malawi, Blantyre, Malawi, [4]Tropical Diseases Research Centre, Ndola, Zambia, [5]Kwame Nkrumah University of Science and Technology, Ku, Ghana, [6]London School of Hygiene & Tropical Medicine, London, United Kingdom, [7]Hubert Kairuki Memorial University, Dar es Salaam, United Republic of Tanzania

Pregnant women are at increased risk of *Plasmodium falciparum* malaria which is associated with increased maternal, foetal and neonatal morbidity and mortality. Thus, malaria-infected pregnant women need prompt and effective treatment. Artemisinin-based combination treatments (ACT) are recommended for pregnant women in their second and third trimesters of pregnancy though information on their safety and efficacy in African pregnant women is limited. A Phase 3, non-inferiority, multicentre, randomized, open-label clinical trial compared the efficacy and safety of four ACTs, namely amodiaquine-artesunate, dihydroartemisinin-piperaquine, artemether-lumefantrine, and mefloquine-artesunate, in women with malaria and in the second or third trimester of pregnancy. A total of 3,423 pregnant women were recruited in Burkina Faso, Ghana, Malawi and Zambia. After being treated with one of the 4 ACTs at day 0, 1 and 2, women were reviewed at days 3, 7, 14, 21, 28, 35, 42, 49, 56 and 63, and whenever they were sick. There were 3 early treatment failures, 2 in Malawi and 1 in Zambia. Eight hundred twenty five women (24.1%) had a recurrent infection during the follow up, 81 (2.4%) of them identified as recrudescences after genotyping. No major safety problems were observed during the follow up. This is the largest trial on ACT use during pregnancy ever done in sub-Saharan Africa. Its preliminary results are reassuring.

1923

EPIDEMIOLOGICAL AND MOLECULAR FEATURES OF DENGUE, ZIKA AND CHIKUNGUNYA CONCURRENT OUTBREAKS IN THE PACIFIC, 2014

Van-Mai Cao-Lormeau[1], Claudine Roche[1], Anita Teissier[1], Eric J. Nilles[2], Didier Musso[1]

[1]Institut Louis Malardé, Papeete - Tahiti, French Polynesia, [2]World Health Organization, Division of Pacific Technical Support, Suva, Fiji

During more than a century dengue has been the only mosquito-borne virus considered as major public health concern for Pacific nations. However, during the past 5 years the epidemiology of arboviruses in the Pacific region has shown terrific changes. The situation gradually switched from the predominant circulation of a single dengue virus (DENV) to active transmission of multiple DENV serotypes and genotypes as observed in French Polynesia from 2013 and in New Caledonia and Fiji since the beginning of 2014. In the mean time, Chikungunya virus (CHIKV) appeared for the first time in New Caledonia with autochtonous cases sporadically reported from 2011 up to 2013, and large outbreaks occurring in Papua New Guinea in 2012, Yap Island in 2013 and in Tonga in 2014. Another unexpected event was the emergence of Zika virus (ZIKV) in French Polynesia at the end of 2013. ZIKV caused in French Polynesia the largest outbreak ever documented, and in a context of active circulation of DENV serotypes 1 and 3. At the beginning of 2014, ZIKV outbreaks also emerged in New Caledonia and Cook Islands. As of April 2014, outbreaks of "dengue-like illnesses" were under investigation in several other Pacific islands suggesting that the situation was evolving from bad to worse. We will describe here the early laboratory investigations that contributed to the identification of the aetiological agents of the outbreaks that recently occurred in the Pacific, notably based on the use of filter paper-spotted serum and saliva collected on cotton swab as a source of viral RNA. Based on phylogenetic data we will discuss how these viruses were introduced from continental regions into the Pacific and how they spread from one Pacific island country to another. We will also discuss the particular features of these outbreaks, notably in the occurrence of unusual clinical manifestations, like observed in French Polynesia during the ZIKV outbreak.

1924

POTENT ANTI-MERS COV (MIDDLE EAST RESPIRATORY SYNDROME CORONAVIRUS) FULLY HUMAN ANTIBODIES FROM TRANSCHROMOSOMIC BOVINES FOR PASSIVE IMMUNOTHERAPY

Thomas Luke[1], Kanakatte Raviprakash[1], Eddie Sullivan[2], Stanley Perlman[3], Jincun Zhao[3], Hua Wu[2], Jin-An Jiao[2], Lisa Hensley[4], Reed Johnson[5], Gabriel Defang[1], Peter Jahrling[4], Maya Williams[1]

[1]Naval Medical Research Center, Silver Spring, MD, United States, [2]SAB Biotherapeutics, Sioux Falls, SD, United States, [3]University of Iowa, Iowa City, IA, United States, [4]Integrated Research Facility, National Institute of Allergy and Infectious Diseases, Fort Detrick, MD, United States, [5]Emerging Viral Pathogens Section, National Institute of Allergy and Infectious Diseases, Fort Detrick, MD, United States

No specific treatments of proven effectiveness for Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infections are currently available. The International Severe Acute Respiratory & Emerging Infection Consortium (ISARIC) identified passive immunotherapy with neutralizing antibodies as a treatment approach that warrants priority study. A platform technology using transchromosomic bovines (Tc-bovines) that produce fully human, antigen specific, polyclonal IgG antibody (Tc-pAb) of all subclasses following immunization has been developed. The quantity of Tc-pAb that can be derived from each animal after plasmapheresis ranges from 150 to 300 grams per month. Purified Tc-pAb for intravenous or intramuscular administration has extremely low quantities of bovine proteins and no evidence of adventitious agents. The Tc-bovine platform can also rapidly produce a diverse repertoire of fully human monoclonal antibodies. Two experimental anti-MERS CoV Tc-pAb immunoglobulins were produced in Tc-bovines hyperimmunized with inactivated whole virion Jordan strain virus (clade A) or a recombinant spike protein derived from an Al-Hasa strain (clade B). Both Tc-pAb immunoglobulins, termed SAB-300 and SAB-301, demonstrated 50% plaque reduction neutralizing antibody titers > 10e4/ml and cross neutralized other MERS-CoV strains. SAB-300/SAB-301 were evaluated in recombinant mice expressing the DPP-4 receptor (5 mg/kg and 25 mg/kg IP as a single dose 12 hours before intranasal challenge) and SAB-300 in marmosets (80 mg/kg in 4 divided doses IV starting 24 hours after intratracheal challenge). Control infected mice had a lung viral titer of ~6.0 $\log_{10}$ PFU/mg through day 5 post inoculation but treated mice approached, or were below, the limit of detection (2.0 $\log_{10}$ PFU/mg) by 24-72 hours and displayed no toxicity. Treated marmosets displayed no toxicity and the clinical/virologic data will be presented. Because of these encouraging pre-clinical findings, an IND application is in development.

1925

CLINICAL STUDIES OF DNA VACCINES FOR HEMORRHAGIC FEVER WITH RENAL SYNDROME

Connie Schmaljohn[1], Jay W. Hooper[1], Drew Hannaman[2], Kristopher M. Paolino[3]

[1]United States Army Medical Research Institute for Infectious Diseases, Fort Detrick, MD, United States, [2]Ichor Medical Systems, San Diego, CA, United States, [3]Walter Reed Army Institute of Research, Silver Spring, MD, United States

Hemorrhagic fever with renal syndrome (HFRS) is endemic in Asia, Europe and Scandinavia, and is caused by infection with the hantaviruses Hantaan (HTNV), Seoul (SEOV), Puumala (PUUV), or Dobrava (DOBV) viruses. We developed candidate DNA vaccines for HFRS expressing Gn and Gc genes of HTNV or PUUV and evaluated them in an open-label, single-center Phase 1 study. Three groups of nine subjects each were vaccinated on days 0, 28 and 56 with the DNA vaccines for HTNV, PUUV, or mixture of both vaccines using the Ichor Medical Systems TriGridTM Intramuscular Delivery System (TDS-IM). All vaccinations consisted of a total dose of 2.0 mg DNA in an injected volume of 1 mL saline. For the combined vaccine, the mixture contained equal amounts (1 .0 mg) of each DNA vaccine. There were no study-related serious adverse events (SAEs). Neutralizing antibody responses were detected in 5/9 and 7/9 of individuals who completed all three vaccinations with the HTNV or PUUV DNA vaccines, respectively. In the combined vaccine group, 7/9 of the volunteers receiving all three vaccinations developed neutralizing antibodies to PUUV. The three strongest responders to the PUUV vaccine also had strong neutralizing antibody responses to HTNV. These results demonstrate that the HTNV and PUUV DNA vaccines delivered by TDS-IM separately or as a mixture are safe. In addition, both vaccines were immunogenic, although when mixed together, more subjects responded to the PUUV than to the HTNV DNA vaccine, suggesting immunological interference. Consequently, we have developed an optimized HTNV DNA vaccine that shows no interference in hamsters when mixed with the PUUV vaccine. A Phase 2a clinical study will be initiated in 2014 to assess dose and schedule with the combined, optimized HTNV and PUUV DNA vaccines. An additional Phase 1 study is being planned to compare intradermal and intramuscular delivery of the mixed DNA vaccines.

1926

AN INEXPENSIVE SYSTEM FOR PRODUCING STRUCTURALLY STABLE REPLICATIVE RNA VIRUS-BASED NANOPARTICLE VACCINES

Yiyang Zhou, Christopher Kearney

Baylor University, Waco, TX, United States

Tropical infectious disease vaccine development requires particular attention to cost-efficiency and minimized storage conditions to be optimally useful in third world countries. In this study, the use of a plant-produced, trans-encapsidated non-pathogenic RNA virus as a vaccine vector addresses these issues. Plant-based manufacturing allows large scale production with greatly reduced expense. Transencapsidation with *Tobacco mosaic virus* (TMV) coat protein provides superior chemical and environmental protection to any RNA sequence containing a TMV encapsidation site. A transencapsidated RNA virus that expresses vaccine antigens in human cells would be highly attractive for vaccine use due to its ability to induce innate immune activation pathways to aid immunogenicity of its antigen payload. To avoid the potential safety concerns using a human virus, we have used the insect RNA virus, *Flock House virus* (FHV), which replicates in both human and plant cells but is not pathogenic to either. The RNA2 of the bipartite FHV genome codes for FHV coat protein and is not necessary for replication. We created an FHV RNA1/eGFP vector and noted strong expression of eGFP in inoculated *Nicotiana benthamiana* plants. This was followed by inserting the TMV encapsidation site into the FHV RNA1 and inoculating plants in combination with a high expression vector derived from *Foxtail mosaic virus* to express TMV coat protein. The production of *in planta* nanoparticles was verified by transmission electron microscopy and these yielded an immune response in vaccinated mice that was superior to that of *in vitro* assembled nanoparticles. In this study, we validated the use of *in planta* encapsidated RNAs as an immune activator in the absence of adjuvants. We can now use this system to create sturdy and inexpensive vaccines for tropical infectious diseases.

1927

SEROPREVALENCE OF NGARI AND BUNYAMWERA VIRUSES IN SELECT PARTS OF RIFT VALLEY AND NORTHERN KENYA

Collins O. Odhiambo[1], Marietjie Venter[2], Robert Swanepoel[2], Rosemary Sang[1]

[1]International Centre for Insect Physiology and Ecology, Nairobi, Kenya, [2]University of Pretoria, Pretoria, South Africa

Ngari and Bunyamwera viruses are among the few mosquito-borne human pathogens in the Orthobunyavirus genus, family Bunyaviridae, associated with febrile illness. Ngari virus has been associated with hemorrhagic fever during Rift Valley fever outbreaks in Africa. Ngari virus is a reassortant virus composed of the S and L segments from Bunyamwera and the M segment from Batai virus. While isolations of both viruses have been made from mosquito and tick vectors in the transmission foci in Kenya, no human serosurveys have been conducted. We report findings from a retrospective serosurvey of febrile ill patients attending three health facilities located in Sangailu, Kotile (in Garissa) and Naivasha in Kenya. Bunyamwera and Ngari virus specific antibodies were detected by plaque reduction neutralization tests in 84 (24.3%) of 345 persons tested; Prevalence rates were 11.9% for Bunyamwera virus and 15.9% for Ngari virus. Multivariable analysis revealed age and location as risk factors for Bunyamwera and Ngari virus infections. Patients presenting with febrile illness in identified endemic regions should be vigorously investigated to determine the public health impact of these infections especially during seasons of high mosquito abundance.

1928

ITAYA VIRUS: A NOVEL *ORTHOBUNYAVIRUS* ASSOCIATED WITH HUMAN FEBRILE ILLNESS IN PERU

Carolina Guevara[1], Jesus Silvas[2], Robert Hontz[1], Wilma Casanova[3], Nikos Vasilakis[2], Douglas Watts[4], Tadeusz Kochel[1], Julia S. Ampuero[1], Hideki Ebihara[5], Patricia V. Aguilar[2]

[1]U.S. Naval Medical Research Unit - 6, Lima, Peru, [2]Department of Pathology, Institute for Human Infections and Immunity, University of Texas Medical Branch, Galveston, TX, United States, [3]Direccion Regional de Salud Loreto, Iquitos, Peru, [4]University of Texas at El Paso, El Paso, TX, United States, [5]Laboratory of Virology, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rocky Mountain Laboratories, Hamilton, MT, United States

The *Orthobunyavirus* genus in the family *Bunyaviridae* comprises more than 170 viruses, at least 30 of them associated with human disease. Caraparu virus, a member of the group C (*Orthobunyavirus* genus), was first isolated from a sentinel monkey in Brazil and subsequently isolated from febrile patients in Bolivia, Brazil, Peru and Trinidad. Febrile surveillance studies conducted by the U.S. Naval Medical Research Center Unit No. 6 identified Caraparu virus (and other group C viruses) as an important cause of febrile illness in the Amazon region of Peru. We conducted genetic analyses of previously uncharacterized bunyavirus strains isolated from febrile patients in Peru, and identified a novel reassortant virus containing the S and L segment of Caraparu virus and the M segment of an unidentified Group C virus. Neutralization test using mouse antisera prepared against the prototype Caraparu strain BeAn 3994 and the novel reassortant virus showed that there was more than a 4-fold difference in titer between these viruses, indicating that the new reassortant was serologically distinct from the prototype Caraparu strain. Serological analyses also confirmed that the novel reassortant was antigenically

distinct from Peruvian Caraparu strains. This new reassortant virus, which we named Itaya virus, was first isolated during 1999 from a 25-year-old male febrile patient in Iquitos, Peru, and subsequently isolated during 2006 from a 59-year-old male febrile patient in Yurimaguas, another city within the Amazon region of Peru. Geographical distance between these two cases indicates that Itaya virus may be widely distributed within the Peruvian Amazon. The recognition of a new *Orthobunyavirus* human pathogen in the Amazon region of Peru reinforces the need to continue and expand viral disease surveillance activities in tropical regions of South America.

1929

MAPPING THE ZOONOTIC NICHE OF EBOLA VIRUS DISEASE IN AFRICA

David Pigott[1], Nick Golding[2], Adrian Mylne[2], Zhi Huang[2], Andrew Henry[2], Daniel Weiss[2], Oliver Brady[2], Moritz Kraemer[2], David Smith[2], Catherine Moyes[2], Samir Bhatt[2], Peter Gething[2], Peter Horby[2], Isaac Bogoch[3], John Brownstein[4], Sumiko Mekaru[4], Andrew Tatem[5], Kamran Khan[3], Simon Hay[2]

[A]Department of Zoology, [1]University of Oxford, Oxford, United Kingdom, [2]University of Toronto, Toronto, ON, Canada, [3]Boston Children's Hospital, Boston, MA, United States, [4]University of Southampton, Southampton, United Kingdom

Ebola virus disease (EVD) is a complex zoonosis that is highly virulent in humans. The largest recorded outbreak of EVD is ongoing in West Africa, outside of its previously reported and predicted niche. We assembled location data on all recorded zoonotic transmission to humans and Ebola virus infection in bats and primates (1976-2014). Using species distribution models these occurrence data were paired with environmental covariates to predict a zoonotic transmission niche covering 22 countries across Central and West Africa. Vegetation, elevation, temperature, evapotranspiration and suspected reservoir bat distributions define this relationship. At-risk areas are inhabited by 22 million people, however the rarity of human outbreaks emphasises the very low probability of transmission to humans. Increasing population sizes and international connectivity by air since the first detection of EVD in 1976 suggest that the dynamics of human-to-human secondary transmission in contemporary outbreaks will be very different to those of the past.

1930

USE OF A NOVEL CHAGAS URINE NANOPARTICLE TEST (CHUNAP) FOR DIAGNOSIS OF CONGENITAL CHAGAS DISEASE

Yagahira E. Castro[1], Robert Gilman[2], Gerson Galdos-Cardenas[2], Lisbeth Ferrufino[3], Gerardo Sánchez[1], Edward Valencia Ayala[1], Lance Liotta[4], Caryn Bern[5]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Johns Hopkins University, Bloomberg School of Hygiene and Public Health, Baltimore, MD, United States, [3]Hospital Universitario Japonés, Santa Cruz, Plurinational State of Bolivia, [4]Center for Applied Proteomics and Molecular Medicine, George Mason University, Manassas, VA, United States, [5]Global Health Sciences, Department of Epidemiology and Biostatistics School of Medicine, University of California San Francisco, CA, United States

Detection of congenital *Trypanosoma cruzi* transmission is considered one of the pillars of control programs of Chagas disease, because 25% of new infections occur by this route with an estimated of 15,000 infected infants per year in Latin America. Current programs to detect congenital Chagas disease in Latin America utilize microscopy early in life and serology after 6 months. These programs suffer from low sensitivity by microscopy and high loss to follow-up later in infancy. We developed a Chagas urine nanoparticle test (Chunap) to concentrate, preserve and detect *T. cruzi* antigens in urine for early, non-invasive diagnosis of congenital Chagas disease. This is a proof-of-concept study to provide an initial indication that Chunap allows for the early diagnosis of congenital Chagas disease. Poly N-isopropylacrylamide nano-particles functionalized with trypan blue were synthesized by precipitation polymerization and characterized with photon correlation spectroscopy. We evaluated the ability of the nanoparticles to capture, concentrate and preserve *T. cruzi* antigens. Urine samples from congenitally infected and uninfected infants were then concentrated using these nanoparticles. The antigens were eluted and detected by Western Blot using a monoclonal antibody against *T. cruzi* lipophosphoglycan. The nanoparticles concentrated *T. cruzi* antigens by 100 fold (western blot detection limit decreased from 50 ng/ml to 0.5 ng/ml). The sensitivity of Chunap in a single specimen at one month of age was 91.3% (21/23, 95% CI: 71.92%-98.68%), comparable to PCR in two specimens at 0 and 1 month (91.3%) and significantly higher than microscopy in two specimens (34.8%, 95% CI: 16.42%-57.26%). Chunap specificity was 96.5% (71/74 endemic, 12/12 non-endemic specimens). Particle-sequestered *T. cruzi* antigens were protected from trypsin digestion. Chunap has the potential to be developed into a simple and sensitive test for the early diagnosis of congenital Chagas disease.

1931

A THERAPEUTIC NANOPARTICLE VACCINE AGAINST *TRYPANOSOMA CRUZI* IN A MOUSE MODEL OF CHAGAS DISEASE

Meagan A. Barry, Qian Wang, Coreen M. Beaumier, Kathryn M. Jones, Brian P. Keegen, Maria Elena Bottazzi, Michael J. Heffernan, Peter J. Hotez

Baylor College of Medicine, Houston, TX, United States

Chagas disease is a neglected tropical disease of great importance in the Americas, with 7-8 million people infected. The causative agent is *Trypanosoma cruzi*, and results in an acute febrile illness that progresses to chronic chagasic cardiomyopathy in 30% of patients. In endemic areas, Chagas disease is the leading cause of cardiovascular death between ages 30-50. Current pharmacological treatments are plagued by significant side effects, poor efficacy, and are contraindicated in pregnancy. There is an urgent need for new treatment modalities. A therapeutic vaccine for Chagas disease has potential advantages that include cost savings, reduced adverse effects, and the potential to be used as a replacement for current therapies or when paired with chemotherapy. Prior work in mice has identified an efficacious *T. cruzi* antigen (Tc24). To elicit a protective cell-mediated immune response to the Tc24 protein, we have utilized a nanoparticle delivery system in conjunction with CpG motif-containing oligodeoxynucleotides (ODN) as an immunomodulatory adjuvant. When tested in a BALB/c mouse model, a dose response study demonstrated a positive relationship between dose of vaccine and Tc24-specific IFN-γ response. Our nanoparticle vaccine, comprised of Tc24 and CpG ODN encapsulated in poly(lactic-co-glycolic acid) (PLGA) nanoparticles, produced the most robust T_H1-mediated $CD8^+$ T cell immune response. When tested for therapeutic efficacy in *T. cruzi* infected BALB/c mice, improved survival was seen in the vaccine group compared to the control groups. Additionally, there was a significant reduction in the number of parasites in the cardiac tissue of the vaccine group compared to the PBS sham vaccine group, indicating protection from parasite-driven cardiac damage. The mice that survived to the end of the study had almost undetectable numbers of parasites in the cardiac tissue. These data demonstrate the immunogenicity and efficacy of a Tc24/CpG ODN nanoparticle vaccine and are convincing evidence for a potential new therapeutic vaccine against Chagas disease.

1932

ASSEMBLING NEW CHEMICAL BOXES AS AN OPEN SOURCE OF STARTING POINTS FOR DRUG DISCOVERY AGAINST KINETOPLASTID PARASITES CAUSING NEGLECTED TROPICAL DISEASES

Julio J. Martin[1], Emilio Alvarez[1], Ana I. Bardera[1], Juan Cantizani[1], Gonzalo Colmenarejo[1], Jose M. Fiandor[1], Albane Kessler[1], Maria P. Manzano[1], Julio Alonso-Padilla[2], Imanol Peña[1], Ana Rodriguez[2]

[1]GlaxoSmithKline, Tres Cantos, Spain, [2]New York University School of Medicine, New York, NY, United States

Neglected Tropical Diseases (NTDs) are a group of infectious diseases categorized by the particular neglect they have suffered in terms of investment in control measures, when compared with malaria and tuberculosis, "the big two" within the diseases. The NTDs encompass a broad range of viral, bacterial, and parasitic infections. Kinetoplastids are a group of flagellated protozoans that include the species *Leishmania* and *Trypanosoma*, some of which are human pathogens with devastating health and economic effect. The most common human diseases caused by kinetoplastids are included within the list of 17 core NTDs declared by WHO. They are human African trypanosomiasis, caused by subspecies of *T. brucei;* Chagas disease, caused by the infection with *T. cruzi*; and various clinical manifestations of leishmaniasis, caused by more than 20 species of *Leishmania*. All NTDs have been categorized as "tool ready," yet also "tool deficient" because many of these tools (i.e. drugs and diagnostics) and implementation strategies are inadequate to achieve the desired goals. New effective, safe, and affordable drugs, preferably oral, are needed. The general neglect that these diseases have encountered by the pharmaceutical industry has meant that basic research findings have not found their way into a drug discovery pipeline. In this paper we present an integral approach to the early drug discovery for the three major kinetoplastid NTDs, i.e. visceral leishmaniasis, Chagas disease and sleeping sickness. The GSK 1.8 million compounds diverse collection has been screened phenotypically against their causative parasites, respectively *L. donovani*, *T. cruzi* and *T. brucei*, using the state-of-the-art methodologies available in high throughput screening. As a result of this effort, three anti-kinetoplastidal boxes of approximately 200 compounds each have been assembled, which represent all the chemical and biological diversity identified and are intended to serve as an open source of starting points for further lead discovery programs.

1933

A FULLY INTEGRATED PARTNERSHIP PERFORMING DRUG DISCOVERY TOWARDS VISCERAL LEISHMANIASIS: PART 1

Paul G. Wyatt

University of Dundee, Dundee, United Kingdom

GSK Kinetoplastid DPU and the Drug Discovery Unit, University of Dundee have formed a partnership to conduct drug discovery within kinetoplastid diseases (Visceral Leishmaniasis, Chagas disease and human African trypanosomiasis). The collaboration, with support from the Wellcome Trust has made significant progress, in building new methods and infrastructure to carry out drug discovery for these parasites. Our advances has resulted in the identification of a lead optimisation series for Visceral Leishmaniasis through phenotypic optimisation. Estimates suggest that Visceral Leishmaniasis worldwide causes 51,000 deaths per year. The current drugs are not fit for purpose, suffering from many issues including poor efficacy and unacceptable levels of toxicity. Part 1, by Paul Wyatt from the Drug Discovery Unit, will describe the transition of a T. brucei GSK3 kinase inhibitor series into a series that fulfils lead optimisation criteria for Visceral Leishmaniasis. This novel series is one of the few reported globally to show oral efficacy in an acute *in vivo* mouse model against Visceral Leishmaniasis. Part 2, by Tim Miles from GSK, will concentrate on the lead optimisation and progression of this series. As a number of issues were highlighted through critical path screening that have been overcome (i.e. solubility and exposure). Hence a discussion of medicinal chemistry strategies to solve these issues within a phenotypic screening setting will be discussed. The current set lead compounds within this series are being evaluated for pre-candidate selection.

1934

CLINICAL EVALUATION OF CL DETECT™ RAPID TEST FOR CUTANEOUS LEISHMANIASIS: PERFORMANCE CHARACTERISTICS WHEN COMPARED TO SMEAR MICROSCOPY AT MULTIPLE TEST SITES

Afif Ben Salah[1], Nabil Bel Haj Hamida[1], Amor Zaatour[1], Adel Gharbi[1], Jihene Bettaieb[1], Wissem Ghawar[1], Amina Khedher[1], Aicha Boukthir[1], Nathalie Ben Messaoud[1], Evelyne Guedri[1], Nathalie Ben Mbarek[1], Nasri Mbarek[1], Rabiaa Harrabi[1], Chadhi Dawahi[1], Hongjing Chen[2], Stan Morkowski[2], James Needham[2], Annabel Tsai[2], Yvonne Stevens[2], Syamal Raychaudhuri[2], Stuart Kahn[2], Janet Ransom[3], Charles Scott[3], Giselle Singer[4], Danielle Baum[4], Shelby Jim On[4], Mark Richardson[4], William McCarthy[5], Max Grogl[5], Mara Kreishman-Deitrick[5], Jeanne Norwood[5], Raymond Houghton[2], Mark Lebwohl[4]

[1]Institut Pasteur de Tunis, Tunis, Tunisia, [2]InBios International Inc., Seattle, WA, United States, [3]Fast-Track Drugs and Biologics, LLC, North Potomac, MD, United States, [4]Icahn School of Medicine at Mount Sinai, New York, NY, United States, [5]Office U.S. Army Medical Materiel Development Activity, Fort Detrick, MD, United States

This study focused on establishing the performance characteristics of the CL Detect™ rapid immunoassay when compared to smear microscopy. The assay is intended for the diagnosis of Cutaneous Leishmaniasis in far-forward, rugged environments. The test is based on the detection of the thiol specific antioxidant protein (TSA, Peroxidoxin) present in amastigotes and promastigotes of L. major and other Leishmania spp. It uses a capture polyclonal antibody to TSA in combination with a gold conjugated monoclonal antibody directed to L. major amastigotes but reactive with TSA. Testing was performed on samples from skin lesions collected with a dental broach device. In each case dipstick reactivity was compared to the lesion parasite load determined by microscopy and quantified using the WHO scale. A total of 168 patients ranging in age from 18-79 years with suspected CL lesions were enrolled with written informed consent at 2 sites endemic for L. major infections in central Tunisia (Sidi Bouzid, Gafsa). 149 were positive by CL Detect™ and microscopy while 16 were negative by both tests and 3 were positive by dipstick but not microscopy. Of these three, 1 was positive by culture. Of the 16 negatives by dipstick and microscopy 2 were positive by culture. In the Icahn School of Medicine, Mount Sinai specificity study, 150 samples were tested by CL Detect™ and microscopy. These included patients ranging in age from 18-92 years with other skin lesions and non-CL infections. In the specificity study 144 of 150 were true negatives for parasites by both CL Detect™ and microscopy for a specificity of 96.0%. Six samples negative by microscopy were low positive by rapid test but negative by microscopy. Cross reactivity studies with other bacteria, parasites, viruses and fungi confirmed specificity for Leishmania spp. Interference, stability and reproducibility studies indicate that CL Detect™ is a robust assay. The pairing of this test with a safe and easy to use drug treatment has the potential to greatly enhance the management of CL patients in far-forward rugged environments.

1935

ACCESS TO DIAGNOSIS AND TREATMENT FOR CHAGAS DISEASE IN THE UNITED STATES: A HEALTH SYSTEMS PERSPECTIVE

Jen Manne-Goehler[1], Michael R. Reich[2], Veronika J. Wirtz[3]

[1]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, United States, [2]Department of Global Health and Population, Harvard School of Public Health, Boston, MA, United States, [3]Boston University Center for Global Health and Development, Boston, MA, United States

Chagas disease, caused by infection with *Trypanosoma cruzi*, is a vector-borne disease with an estimated 300,000 cases in the United States (US). Screening of blood donors for infection with *T. cruzi* was started in 2007 in the US. Currently, benznidazole and nifurtimox are available to treat this infection through direct request from the US Centers for Diseases Control and Prevention (CDC) under compassionate use protocols. This study analyzed epidemiological trends in diagnosis and treatment for Chagas disease in the US and assessed national and state barriers to access. Data on the distribution of cases of Chagas disease identified in blood donors and drug releases were obtained from the AABB and CDC respectively. Semi-structured in-depth interviews were conducted with 30 key informants at the national level and in 6 high-burden states (CA, FL, VA, NY, MA, and TX) where treatments were provided. Interview responses were analyzed according to the health system's dimensions of regulation, financing, payment, organization, and persuasion. Data indicate that 1,908 cases were identified in the blood donation system from 2007-2013 and that CDC provided 422 courses of benznidazole or nifurtimox during this period. Interview data revealed that local ad-hoc procedures were used by individual physicians with an interest in the disease to increase access to medicines for Chagas disease, especially through cross-financing of patient care activities using grants and donations. The primary barriers to access at the national level include limited diagnostic and institutionalized referral and care processes (Organization), lack of financing for patient care activities in most states (Financing), and limited awareness and training among physicians and patients (Persuasion). This study demonstrates that access to treatment for Chagas disease in the US is limited. The lack of licensing for the two medications used in treatment was only one of several barriers to access, highlighting the need for a health systems perspective when scaling up access to these essential medicines.

1936

IMMUNOGENICITY OF *TRYPANOSOMA CRUZI* VACCINE CANDIDATE ANTIGENS TSA-1 AND TC24 IN MEXICAN CHAGASIC PATIENTS

Liliana Villanueva-Lizama, Amaru Aguilar-Cetina, Vladimir Cruz-Chan, Maria Jesus Ramirez-Sierra, Miguel Rosado-Vallado, Eric Dumonteil

Universidad Autonoma de Yucatan, Merida, Yucatan, Mexico

Chagas disease affects 8-10 million persons worldwide, and at least 1-2 millions in Mexico. Current drugs have a limited efficacy and the development of a preventive and/or therapeutic vaccine is an important goal. Based on previous work in mouse and dog models, trypomastigote surface antigen-1 (TSA-1) and 24-kDa-trypomastigote secretion/excretion antigen (Tc24) are good candidates for a vaccine against *Trypanosoma cruzi*. We evaluated here the recall immune response against these vaccine antigens in Chagas disease patients, as a first step to assess their immunogenicity in humans. We used peripheral blood mononuclear cells (PBMC) from chagasic patients (n=8) and healthy controls (n=8) that were stimulated *in vitro* with TSA-1 and Tc24 recombinant antigens. After 120 hours of stimulation, we evaluated cell proliferation, identified CD4+ and CD8+ memory cell subpopulations, and IFN-gamma and IL-10-producing cells by flow cytometry. We observed a specific proliferative response to TSA-1 and to a lesser extent to Tc24, with a central memory T cell phenotype and antigen-specific INF-gamma and IL-10 production in several Chagas disease patients. Additional patients and controls will be enrolled until September 2014. These preliminary results suggest that the selected antigens are immunogenic in humans and may thus be good candidates for further development of a Chagas disease vaccine

1937

EARLY IL-10 PRODUCTION BY CD4+ T CELLS IN THE SKIN IS FUNCTIONALLY SUPPRESSIVE

David Sanin, Catriona T. Prendergast, Adrian P. Mountford

Centre for Immunology and Infection, Department of Biology, University of York, York, United Kingdom

Communities in endemic areas affected by Schistosomiasis, a parasitic disease caused by *Schistosoma mansoni*, are repeatedly infected when they come into contact with contaminated water. *S. mansoni* cercariae penetrate the skin and elicit a strong inflammatory response, which after repeated exposure becomes both exacerbated and skewed towards type 2 immunity, with high levels of IL-10, IL-4, eosinophilia and alternative activation of macrophages. In compass with this phenomenon at the site of infection, local skin draining draining lymph node (sdLN) cells progressively lose their ability to respond to *S. mansoni* cercarial antigens, becoming unable to proliferate or produce cytokines. Our evidence shows that the effect multiple infections have on sdLN responsiveness is mediated by IL-10, which is found at significantly higher levels in the skin after repeated exposure to the parasite. sdLN cells from IL-10 deficient mice retained their ability to respond to schistosomula antigens, while the immune response in the skin was significantly more pro-inflammatory. After the initial exposure to the parasite, $CD4^+$ T cells and $F4/80^+MHC\text{-}II^{high}$ monocytes produced most of the IL-10 in the skin. Strikingly, $CD4^+$ T cells in the skin made IL-10 as early as day 1 after the initial exposure. This initial response was directed against commensal antigens that would penetrate the skin during cercariae invasion. However, by day 4 after the first exposure, non-regulatory $CD4^+$ T cells respond to *S. mansoni* antigens, expanding considerably after multiple infections and accounting for most of the detected IL-10. Furthermore, IL-10 producing $CD4^+$ T cells from the skin have the ability to inhibit the proliferation of sdLN $CD4^+$ cells. In summary, $CD4^+$ T cells in the skin produce IL-10 and prevent cells in the lymph nodes from responding to repeated infections with the parasite, whilst they contribute to a type 2 immune response environment in the skin. Skin commensals are partly responsible for this type of response, as they penetrate the skin when *S. mansoni* cercarieae invade the tissue.

1938

A CENTRAL ROLE FOR TYPE I IFN IN THE INDUCTION OF TH2 RESPONSES BY DENDRITIC CELLS

Lauren Webb[1], Rachel J. Lundie[2], Jessica G. Borger[2], Peter C. Cook[1], Sheila L. Brown[1], Lucy H. Jones[3], Alexander T. Phythian-Adams[1], Andrew S. MacDonald[1]

[1]Manchester Collaborative Centre for Inflammation Research, University of Manchester, Manchester, United Kingdom, [2]Institute of Inflammation and Infection Research, University of Edinburgh, Edinburgh, United Kingdom, [3]Institute of Inflammation and Infection Research, University of Edinburgh, Edinburgh, United Kingdom

Although dendritic cells (DCs) are critical for induction of Th2 immunity against helminths or allergens, relatively little is known about how they become activated and function in response to Th2-polarizing antigens. We have discovered a previously unrecognized role for Type I IFN (IFN-I) in the optimal activation and function of DCs following exposure to strongly Th2-polarizing antigens from the parasitic helminth *Schistosoma mansoni*. To date, IFN-I has primarily been associated with anti-viral immunity, and its role in Th2 settings is currently unclear. DCs lacking the IFN-I receptor displayed a dramatically impaired ability to induce Th2 cytokines *in vivo*, but unimpaired ability to support Th2 polarization *in vitro*. Further, Th2-promoting DCs depended on IFN-I signaling for efficient migration to the draining LN. We are now investigating whether IFN-I is also required for effective localization within the draining LN and interaction with LN-

resident T cells. Together, our data suggest a key role for IFN-I to enable Th2 induction by DCs against helminths *in vivo*. Future work will address the wider role of IFN-I in Th2 inflammation, including during helminth infection, and the activation of allergic responses in the airways.

1939

VSG-SEQ: A QUANTITATIVE METHOD FOR TRACKING THE *IN VIVO* DYNAMICS OF ANTIGENIC VARIATION IN *TRYPANOSOMA BRUCEI*

Monica Mugnier, George Cross, Nina Papavasiliou

The Rockefeller University, New York, NY, United States

Using a repertoire of over 2000 different variant surface glycoprotein (VSG) genes within its genome, *Trypanosoma brucei,* the causative agent of African sleeping sickness, changes its dense VSG surface coat to avoid detection by the immune system of its mammalian host. The dynamics of antigenic variation in *T. brucei* during an infection, however, are poorly understood. How many variants appear over the course of an infection? Is there a pattern to VSG expression over time? Although some of these questions have been broached using Sanger sequencing of VSG cDNA, technical limitations have prevented a high-resolution, quantitative study of VSG expression during *T. brucei* infection. Here we present VSG-seq, the first method for quantitatively examining the diversity of expressed VSGs in a population of trypanosomes, isolated either from culture or from blood. This next-generation sequencing approach requires very little input material and is quite sensitive, detecting VSGs expressed on less than 0.1% of a population of trypanosomes. Using samples isolated from mouse infections, expressed VSG sequences can be assembled accurately *de novo*, demonstrating that this approach can be used for the high-resolution study of VSG expression in any strain of *T. brucei,* whether in the lab or in the field. We have used VSG-seq to study the kinetics of VSG populations throughout *T. brucei* infections. These studies reveal more complex switching dynamics than previously expected and hint at the possibility of new mechanisms for increasing antigenic diversity *in vivo*.

1940

BLOODSTREAM FORM *TRYPANOSOMA BRUCEI* MEMBRANE NANOTUBES AND EXTRACELLULAR VESICLES MEDIATE INTERCELLULAR INTERACTIONS AND HOST ANEMIA

Anthony Szempruch[1], S. E. Sykes[1], J. M. Harrington[1], A. C. Becker[1], A. Gartrell[1], E. Arigi[2], I. C. Almeida[2], S. L. Hajduk[1]

[1]Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA, United States, [2]Department of Biology, University of Texas El Paso, El Paso, TX, United States

Trypanosoma brucei cycles between an insect vector and a mammalian host, causing human African sleeping sickness and Nagana in cattle. We identified extracellular vesicles (EVs) from wild type bloodstream form (BF) cells by electron microscopy. Purified vesicles have been shown to propagate a distinctive morphological phenotype when added to wild type cells. Fluorescently labeled EVs bind to the trypanosome flagellar pocket and are subsequently endocytosed. We have developed an RNAi cell line that can be induced to produce an excess of EVs. When used in transwell separation experiments, these cells showed EVs mediate phenotype transfer. Proteomic analysis of EVs from both wild type and RNAi cells revealed ~50 shared proteins. In addition, we observe that purified EVs are capable of membrane fusion and transferring variant surface glycoprotein to human red blood cells. This fusion and protein transfer alters the physical properties of the red blood cell membrane, potentially leading to anemia seen during infection. Imaging of *T. brucei* cells reveal the formation of long membrane nanotubes at the posterior end of the cells that are able to bind other trypanosomes. These membrane nanotubes originate from budding of the flagellar membrane and form a helical wrapping structure that resembles "beads on a string." These "beads" closely resemble the structure of cell-associated EVs. In addition, live cell imaging suggests that these nanotubes can disassociate into what appear to be free vesicles. We hypothesize that membrane nanotubes are the structures with which trypanosomes produce EVs. This demonstrates that *T. brucei* is capable of cellular communication and may have significant impact to understanding infection, immune evasion, and differentiation of this parasite.

1941

MONOCYTE-DERIVED ALTERNATIVELY ACTIVATED MACROPHAGES RETAIN PLASTICITY AFTER ACTIVATION AND DIFFERENTIATION

Natasha Girgis, Uma Mahesh Gundra, Michael A. Gonzales, P'ng Loke

New York University School of Medicine, Department of Microbiology, New York, NY, United States

Alternatively activated macrophages (AAM) induced by type 2 responses promote clearance of helminth parasites, wound healing, and tissue remodeling. AAM can accumulate through recruitment and differentiation of monocytes, or through proliferation of tissue resident macrophages. We have recently shown that AAM derived from monocytes ($_{mono}$AAM) are phenotypically and functionally distinct from AAM that arise from tissue macrophages ($_{tiss}$AAM). $_{tiss}$AAM are F480^{high} and express the mitochondrial protein Ucp1, but low levels of MR1 and PDL2, while $_{mono}$AAM were F480^{int} and expressed high levels of MR1 and PDL2. Here, we find that $_{mono}$AAM recruited to sites of inflammation remain plastic after activation and differentiation. In short-term transfer experiments, $_{mono}$AAM donor cells transferred into naïve recipients retain expression of MR1, but lose expression of PDL2. In long-term transfer experiments, $_{mono}$AAM can convert to F4/80^{high} macrophages with a phenotype similar to $_{tiss}$AAM. Hence, $_{mono}$AAM remain plastic after activation, which is dependent on Stat6 signaling in donor AAM, as well as accessory cells in the recipient mice. We have also found that AAM in the hepatic granulomas of mice infected with the parasitic helminth *Schistosoma mansoni* accumulate primarily through the recruitment of inflammatory monocytes. Future experiments will determine if these $_{mono}$AAM will further adopt the phenotype of $_{tiss}$AAM in the livers of infected mice after long-term residence in the granulomas

1942

METABOLIC REGULATION OF TYPE 2 IMMUNITY CONTROLS TISSUE REPAIR

Wildaliz Nieves, Taylor Oniskey, Li-Yin Hung, De'Broski R. Herbert

Division of Experimental Medicine, Department of Medicine, University of California at San Francisco, San Francisco, CA, United States

Host metabolism is profoundly affected by gastrointestinal (GI) nematodes. Many GI nematodes feed upon the tissues and blood of their hosts, resulting in anemia, malnutrition, and generalized immunosuppression. It is debatable whether these features of worm infection are due to parasite and/or host-derived factors. In this study, we investigated whether adenosine monophosphate kinase (AMPK) controlled the outcome of GI nematode infection through controlling the inflammatory response. AMPK is a heterotrimeric enzyme complex of $\alpha\beta\gamma$ subunits that restores cellular energy through oxidative phosphorylation. Given that AMPK activity is regulated by phosphorylation of the catalytic α subunit, we generated CD11c^{Cre} x AMPKα1$^{flox/flox}$ (DC-AMPK$^{-/-}$) mice to study the importance of AMPK in alveolar macrophage and dendritic cell function. DC-AMPK$^{-/-}$ mice infected with the hookworm *Nippostrongylus brasiliensis* (*N.b.*) generated abnormal Type-2 immune responses and failed to regenerate areas of hookworm-damaged tissue 9 days post-primary infection. In comparison to littermate controls, DC-AMPK$^{-/-}$ mice were unable to generate intestinal goblet cell metaplasia and failed to expel adult worms from the intestine. Moreover, *N.b.*-induced lung injury was more severe in DC-AMPK$^{-/-}$ mice and the restoration of pulmonary function was significantly delayed compared to controls. Dysregulated responses generated in DC-AMPK$^{-/-}$ mice were associated with increased Type-1 responses (IL-12, iNOS), greater numbers of T_H17 cells, and defects in the generation of alternatively activated macrophages. Taken together, our data are consistent with an

important role for host metabolism in shaping inflammatory responses during helminth infection. Thus, AMPK activity within myeloid antigen presenting cells regulates host protection against GI parasites.

1943

THE *TOXOPLASMA* DENSE GRANULE PROTEINS GRA17 AND GRA23 MEDIATE THE MOVEMENT OF SMALL MOLECULES BETWEEN THE HOST AND THE PARASITOPHOROUS VACUOLE

Dan Gold[1], Aaron D. Kaplan[2], Agnieszka Lis[2], Glenna C. Bett[2], Emily E. Rosowski[1], Kimberly M. Cirelli[1], Alexandre Bougdour[3], Saima M. Sidik[4], Josh R. Beck[5], Sebastian Lourido[6], Pascal F. Egea[7], Peter J. Bradley[5], Mohamed-Ali Hakimi[3], Randall L. Rasmusson[2], Jeroen P. Saeij[1]

[1]Massachusetts Institute of Technology, Department of Biology, Cambridge, MA, United States, [2]Physiology and Biophysics, The State University of New York, University at Buffalo, Buffalo, NY, United States, [3]Centre National de la Recherche Scientifique, UMR5163, Laboratoire Adaptation et Pathogénie des Microorganismes, Grenoble, France, [4]Whitehead Institute for Biomedical Research, Cambridge, MA, United States, [5]Department of Microbiology, Immunology and Molecular Genetics, University of California Los Angeles, Los Angeles, CA, United States, [6]Université Joseph Fourier, Grenoble, France, [7]Department of Biological Chemistry, University of California Los Angeles David Geffen School of Medicine, Los Angeles, CA, United States

Toxoplasma gondii is a widespread protozoan pathogen in the phylum Apicomplexa that resides intracellularly within a parasitophorous vacuole (PV) that is selectively permeable to small molecules through an unknown mechanism. We have identified GRA17 as a novel *Toxoplasma*-secreted protein, which localizes to the parasitophorous vacuole membrane (PVM) and is conserved across PV-residing apicomplexans. GRA17 mediates the passive transport of small molecules across the PVM. The PVs of GRA17-deficient parasites have aberrant morphology, reduced permeability to small molecules, and structural instability. GRA17-deficient parasites proliferate slowly and are avirulent in mice. GRA17 functions synergistically with a related protein, GRA23. Exogenous expression of GRA17 or GRA23 alters the membrane conductance properties of *Xenopus* oocytes in a manner consistent with a large non-selective pore. GRA17 and GRA23 provide the first molecular basis to explain the PVM permeability to small molecules.

1944

QUANTIFYING LABILE HEME IN LIVE MALARIA PARASITES USING NOVEL FRET BIOSENSORS

James Abshire, Christopher J. Rowlands, Peter T. So, Jacquin C. Niles

Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, United States

The heme cofactor is centrally important in malaria parasite physiology. On one hand, it is a byproduct generated in large quantities during parasite-mediated hemoglobin degradation during the blood stage of malaria infection. Parasites must detoxify excess heme to limit oxidative damage, and do so by polymerizing liberated heme into nonreactive hemozoin. Several successful antimalarial drugs such as the aminoquinolines inhibit this process, presumably leading to the toxic accumulation of labile heme, although this has not been directly demonstrated or quantified. On the other hand, the parasite genome encodes a complete heme biosynthetic pathway that is essential for other stages of parasite development. This suggests heme is a necessary cofactor that is toxic in excess. Despite the central role of heme to parasite metabolism and its link to antimalarial drug potency, little is known about heme dynamics in normal parasite physiology or how these dynamics change under stresses imposed by heme-interacting drugs. To address these questions, we have developed and characterized a family of novel, genetically-encoded FRET biosensors for quantifying labile heme in live parasites. In vitro spectroscopic characterization of the purified protein sensors demonstrates their ability to reversibly bind heme, and to exhibit significant heme-dependent changes in FRET. Our studies with blood-stage parasite lines expressing these biosensors indicate that micromolar concentrations of labile heme are maintained in the parasite cytosol throughout development. Furthermore, exposure to chloroquine, but not pyrimethamine, leads to accumulation of cytosolic labile heme, thus directly linking heme dysregulation to the in situ effects of aminoquinolines. We believe these studies will advance our understanding of how heme perturbation is linked to antimalarial drug potency, and help to mechanistically inform future drug development efforts.

1945

CONDITIONAL EXPRESSION OF PFRIPR CONFIRMS ITS ESSENTIALITY FOR PLASMODIUM *FALCIPARUM* MEROZOITE INVASION INTO HUMAN ERYTHROCYTES

Alan Yap, Alan F. Cowman

Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia

Plasmodium falciparum merozoites actively invade human erythrocytes during blood stage malaria, the stage that causes clinical symptoms. Invasion of erythrocytes is a complex process that involves a cascade of protein-protein interactions between merozoite ligands and erythrocyte receptors. To date, many merozoite ligands have been described as essential based on the inability to knockout the proteins *in vitro*. One of these is *P. falciparum* Rh5 interacting protein (PfRipr). Here we present evidence for the efficient knockdown of PfRipr using a dimerizable Cre recombinase (DiCre) system. PfRipr is an essential invasion protein that forms a complex with *P. falciparum* reticulocyte binding-like homologues 5 (PfRh5). Previous studies have shown that the PfRipr/PfRh5 complex plays a critical role in merozoite attachment and invasion as anti-PfRipr antibodies block merozoite invasion. We generated parasites expressing DiCre, which is activated by the addition of rapamycin leading to the deletion of *Pfripr*. Knockdown of gene and protein levels up to 90% within one cycle of the blood stage (about 48 hours) was achieved. This led to a growth reduction in the following cycles, which relates to a reduction in invasion efficiency as determined by flow cytometry, live-imaging and superresolution microscopy. In summary, conditional regulation of PfRipr confirms the essential role of this protein and further elucidates its functions.

1946

HSP101/PTEX MEDIATES EXPORT OF DIVERSE MALARIA EFFECTOR PROTEINS INTO THE HOST ERYTHROCYTE

Josh Beck, Vasant Muralidharan, Anna Oksman, Daniel E. Goldberg

Washington University School of Medicine, St. Louis, MO, United States

To mediate its survival and virulence the malaria parasite *Plasmodium falciparum* exports hundreds of proteins into the host erythrocyte. To enter the host cell, exported proteins must cross the parasitophorous vacuolar membrane (PVM) within which the parasite resides, but the mechanism remains unclear. A putative Plasmodium translocon of exported proteins (PTEX) has been suggested to be involved for at least one class of exported proteins; however, direct functional evidence has remained elusive. Here we show that export across the PVM requires heat shock protein 101 (HSP101), a ClpB-like AAA+ ATPase component of PTEX. Using a chaperone auto-inhibition strategy, we achieved rapid, reversible ablation of HSP101 function, resulting in a nearly complete block in export with substrates accumulating in the vacuole in both asexual and sexual parasites. Surprisingly, this block extended to all classes of exported proteins, revealing HSP101-dependent translocation across the PVM as a convergent step in the multi-pathway export process. Under export-blocked conditions, association between HSP101 and other components of the PTEX complex was lost while association with exported substrates was maintained, suggesting that HSP101 first recognizes proteins destined for export before feeding them into the translocon. Our results demonstrate an essential and universal role for HSP101 in protein export and provide strong evidence for PTEX function in protein translocation into the host cell.

The number(s) following author name refers to the abstract number.

A

Abstract Author Index

The number(s) following author name refers to the abstract number.

The number(s) following author name refers to the abstract number.

B

Abstract Author Index

The number(s) following author name refers to the abstract number.

The number(s) following author name refers to the abstract number.

C

Abstract Author Index

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.

D

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.

E

F

The number(s) following author name refers to the abstract number.

G

Abstract Author Index

The number(s) following author name refers to the abstract number.

The number(s) following author name refers to the abstract number.

H

Abstract Author Index

The number(s) following author name refers to the abstract number.

The number(s) following author name refers to the abstract number.

I

J

Abstract Author Index

The number(s) following author name refers to the abstract number.

K

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.

L

Abstract Author Index

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.

M

Abstract Author Index

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.

N

Abstract Author Index

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.

The number(s) following author name refers to the abstract number.

P

Abstract Author Index

The number(s) following author name refers to the abstract number.

Q

The number(s) following author name refers to the abstract number.

R

Abstract Author Index

The number(s) following author name refers to the abstract number.

S

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.

T

The number(s) following author name refers to the abstract number.

U

V

Abstract Author Index

The number(s) following author name refers to the abstract number.

W

Abstract Author Index

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.