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CONSIDERABLE PROPORTION OF *LEISHMANIA BRAZILIENSIS* RRNA MOLECULES ARE POLYADENYLATED

Marlene Jara Portocarreo, Jorge Arevalo

Instituto de Medicina Tropical Alexander von Humboldt, Lima, Peru

Leishmania parasites are ancestral eukaryotes with unusual characteristics like polycistronic transcription and RNA trans-splicing. Like other eukaryotes, their RNA ribosomal genes are tandemly repeated and transcribed by RNA polymerase I. Unlike other eukaryotes, *Leishmania* ribosomes have rRNA molecules of 18S, 5.8S, and 28S, with the latter one being split into six rRNAs (α.γ, β, δ, ζ and ε). The polyadenylation is a post-transcriptional process well known for mRNA but scarcely reported for rRNA. Our previous work on *L. braziliensis* and *L. donovani* demonstrated that at least the rRNA 28S ε undergo the polyadenylation process and that its relative abundance varies in *Leishmania* promastigote and amastigote stages. To determine if all rRNA gene subunits are subjected to polyadenylation, we evaluated the 18S rRNA, 5.8S rRNA and all the subunits homolog to 28S rRNA at stationary and logarithmic phase promastigotes of the *L. braziliensis* strain MHOM/BR/75/M2904. We found that all the rRNA subunits were polyadenylated. Moreover, we quantified the absolute amount of polyadenylated and non-polyadenylated rRNA of the sub-units 18S, 5.8S and 28S α by Reverse Transcription-Real time quantitative PCR. In the logarithmic promastigotes, the percentage of polyadenylated rRNA 18S, rRNA 5.8S and rRNA 28S α were 0.378 ± 0.02 (mean ± standard deviation), 4.55 ± 0.43 and 13.86 ± 0.95, respectively. The stationary promastigotes had higher percentages of polyadenylated rRNA 18S (0.704 ±0.29, *P*=0.064) and rRNA 5.8S (5.69 ± 0.28, *P*=0.045) than the logarithmic promastigotes, whereas the 28S α did not show any significant differences between log and stationary promastigotes. These findings confirm a remarkable fact of *Leishmania* rRNA gene expression (also present in *L.amazonensis*, data not shown) and it is related to the parasite growth. The biological role of this phenomenon remains unknown but its wide conservation in the genus *Leishmania* indicates it is an important one.

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PHENOTYPIC CHARACTERISTICS OF ACUTE CHAGASIC MYOCARDITIS AMONG C57 AND BALB/C MICE

Andrés F. Henao-Martínez, Anne H. Agler, Timothy A. McKinsey, David A. Schwartz, Ivana V. Yang

University of Colorado Denver, Aurora, CO, United States

Chagasic disease is a notable neglected tropical disease with high morbidity in Latin America and among immigrants to the US. The primary mechanism of mortality is cardiomyopathy and sudden death. Acute chagasic myocarditis is consistently found in acute infections but little is known about its contribution to chronic forms of cardiomyopathy and what host factors play a role in acute myocarditis. The aim of this study was to phenotypically characterize two strains of mice with differential susceptibility to acute chagasic infection and correlate strain phenotypes with heart tissue gene expression. Laboratory mouse Tulahuen strain of *Trypanosoma cruzi* was grown in 3T3 fibroblast cell culture and tissue-derived trypomastigotes (TCT) were harvested from supernatant. C57 and Balb/c mice were injected intraperiotneally with 0 or 150-200 TCT. Weekly, mice were weighed and *parasitemia* was monitored via retro-orbital blood sample. At 4 weeks Brain natriuretic peptide (BNP) and Troponin were measured in plasma and echocardiograms were obtained. 4-week mortality was 56.3% and 12.5% for Balb/c and C57 (p=0.009), respectively. Infected Balb/c mice lost more weight than infected C57 mice (p=0.018). Parasitemia peaked at 2 weeks, but was not significantly different between strains due to high variation in counts: 500,781 ± 866,464 (Balb/c) vs. 140,625 ± 280,606 (C57) parasites/ml (p=0.12). For infected mice, BNP and troponin levels were not significantly different between strains, but BNP differed from uninfected mice. Echocardiograms demonstrated differences in heart rate in BALB/c vs. C57 mice: 413 vs. 476 bpm, (p=0.0001) and stroke volume: 31.9 ± 9.3 vs. 39.2 ± 5.5 µl (p=0.03); therefore in cardiac output: 13.1 ± 3.5 vs. 18.7 ± 3.2 µl/min (p=0.002). There are relevant susceptibility and hemodynamic differences between these strains of mice during acute chagasic infection. Further characterizations of heart tissue histopathology, immunohistochemistry and gene expression will investigate possible host factor determinants for acute chagasic myocarditis.

1250A

QUANTITATIVE KDNA ASSESSMENT DURING TREATMENT OF MUCOSAL LEISHMANIASIS AS A POTENTIAL BIOMARKER OF OUTCOME

Marlene Jara[1], Braulio M. Valencia[1], Milena Alba[1], Vanessa Adaui[1], Jorge Arevalo[1], Alejandro Llanos-Cuentas[1], **Andrea K. Boggild**[2]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]University of Toronto, Toronto, ON, Canada

Mucosal leishmaniasis (ML) is a disfiguring manifestation of infection with *Leishmania* (Viannia) *spp.* As there is no known biomarker of treatment outcome in ML, we evaluated the concentration of kinetoplast minicircle DNA (kDNA) by cytology brush quantitative PCR before, during, and after treatment of ML in Peruvian patients. ML lesions were sampled by cytology brushes for quantitative PCR at enrolment, days 14 and 21_28 of therapy, and 3-, 6-, or 12-mos after treatment. Parasite concentration in tissue was correlated to demographic, clinical, and parasitologic factors. Twenty patients completed follow-up: 12 men and 8 women, with median age of 37 yrs (range 18_78 yrs). Fifteen patients were treated with sodium stibogluconate, and 5 with amphotericin B. Cure was achieved in 17 patients, while 2 patients failed multiple courses of therapy. Clinical outcome is unknown in 1 patient. Mean parasite load (PL) at enrolment was 85,614.8 ± 60,427.3 parasites per ug of tissue DNA (par/ug tDNA). Three patterns of quantifiable kDNA during therapy and follow-up emerged: pattern 1 (N=10) was characterized by a mean PL of 170,867 ± 117,482.6 at enrolment, with sequential decline in PL during and after therapy until kDNA was undetectable. Pattern 2 (N=4) was characterized by mean PL of 566.4 ± 306.4 at enrolment, with clearance of detectable kDNA by D14 of treatment, followed by an increased PL by D21-28 of treatment to 80.4 ± 32.1 par/ug tDNA. Pattern 3 (N=6) was characterized by mean PL of 226.7 ± 116.1 at enrolment, with clearance of detectable kDNA during treatment, followed by increased PL by 6-mos follow-up to 36.6 ± 13.1 par/ug tDNA. Both patients who failed treatment demonstrated Pattern 1. Patterns 2 and 3 were associated with granulomatous inflammation (p=0.02). Younger age (33.5 vs. 64 yrs, p=0.10) and shorter ML duration (20.5 vs. 48 mos, p=0.11) are potentially correlated to sequential clearance (pattern 1). Baseline PL, sex, exposure duration, lesion number, and ML location were not correlated to pattern of PL. We have demonstrated that the concentration of parasite kDNA in ML can be quantified by cytology brush sampling and quantitative PCR during and after treatment. Interim analysis demonstrates 3 distinct patterns of PL during and after treatment, which warrant further investigation. Granulomatous inflammation may predict rebound of PL during or after treatment, though the clinical significance of this rebound is presently unknown.

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COMPARISON OF TWO COMBINATION PARASITE LACTATE DEHYDROGENASE-BASED RAPID TESTS FOR THE DIAGNOSIS OF MALARIA DUE TO *PLASMODIUM KNOWLESI* AND OTHER *PLASMODIUM* SPECIES IN SABAH, MALAYSIA

Matthew J. Grigg[1], T. William[1], B. E. Barber[1], U. Parameswaran[1], T. W. Yeo[2], N. M. Anstey[2]

[1]Queen Elizabeth Hospital, Kota Kinabalu, Sabah, Malaysia, [2]Menzies School of Health Research and Charles Darwin University, Darwin, Australia

Plasmodium knowlesi human infection has been reported throughout South-East Asia, and is the most common cause of severe malaria in parts

of Borneo. Microscopic misdiagnosis is common, and may impact prompt initiation of treatment shown to improve mortality outcomes. Previous studies have shown cross-reactivity of *P. knowlesi* with parasite lactate dehydrogenase monoclonal antibodies used to detect *P. falciparum* and *P. vivax*. Our initial evaluation of rapid diagnostic tests (RDTs) has not demonstrated sufficient sensitivity for *P. knowlesi*, and no specific antibody for *P. knowlesi* has been developed. At both tertiary and district referral sites in Sabah, Malaysia, we prospectively evaluated two combination RDTs for the diagnosis of uncomplicated and severe malaria. Firstly with a pan-*Plasmodium* parasite lactate dehydrogenase (pan-pLDH) and *P. falciparum* specific parasite lactate dehyrogenase (PfLDH) RDT (Optimal-IT). Secondly with a non-*P. falciparum* pan-parasite lactate dehydrogenase (VOM), and *P. falciparum* histidine-rich protein-2 (HRP2) RDT (Carestart). Among 250 patients hospitalised with PCR-confirmed *P. knowlesi*, *P. falciparum* and *P. vivax* monoinfection, the pre-treatment sensitivity of the pan-pLDH test for each species was 36% (49/137; 95% confidence interval [CI] 28 to 44%), 75% (63/84; CI 64 to 84), and 83% (24/29; CI 64 to 94) respectively. The PfLDH test sensitivities were 33% (45/137; CI 25 to 41), 77% (65/84; CI 67 to 86) and 14% (4/29; CI 4 to 32) respectively. The VOM component was the most sensitive test for both uncomplicated (44%; 60/137; CI 35 to 53) and severe (79%; 15/19; CI 54 to 94) *P. knowlesi* malaria but remained clinically insufficient. More sensitive RDTs or alternative molecular diagnostic tools are needed in areas of *P. knowlesi* endemicity.

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COMPARATIVE ANALYSIS OF MALARIA INFECTIONS BY NESTED PCR USING A POOLING STRATEGY ON DRIED BLOOD SPOTS AND PLACENTAL HISTOLOGY IN MICROSCOPY-NEGATIVE MALAWIAN WOMEN ON IPTP

Zhiyong Zhou[1], Julie R. Gutman[1], Dyson Mwandama[2], Doreen Ali[3], Don Mathanga[2], Jacek Skarbinski[1], Ya Ping Shi[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]University of Malawi College of Medicine, Blantyre, Malawi, [3]National Malaria Control Program, Ministry of Health, Lilongwe, Malawi

Malaria infection in pregnant women on intermittent preventive treatment in pregnancy (IPTp) often presents low parasite densities at delivery and this poses a great diagnostic challenge. In this study, a nested polymerase chain reaction (nPCR) assay for the 18S rRNA gene of *Plasmodium falciparum* was conducted to detect malaria infection in microscopy-negative pregnant women at delivery from an IPTp effectiveness study conducted in Malawi. A sample pooling strategy was developed for screening malaria infection using dried blood spots samples (DBSs) collected from placenta or periphery at delivery. Considering a known malaria prevalence of 7.6% by microscopy in pregnant women at delivery, histologic results were used to stratify the 619 available microscopy-negative samples into sample pools. Each sample pool contained 4 DBSs from histology-positive samples or 10 DBSs from histology-negative samples prior to DNA extraction for first round of nPCR screening. For those nPCR-positive pools, DBSs were then individually extracted and a second round of nPCR assay was performed. Overall, of 619 microscopy-negative DBSs, 179 (28.9%) were positive by histology and 52 (8.4%) were positive by nPCR. Among the histology-positive samples, 39 (21.8%) had active infection (acute and chronic) and 140 (78.2%) had past infection. Using the histology results as a reference, 71.8% women were nPCR-positive in the active infection group, 7.1% were nPCR-positive in the past infection group, and 3.2% were nPCR-positive in histology-negative group. In conclusion, histology diagnosis detected more malaria infection, but nPCR combined with a proper sample pooling strategy is still a practical and sensitive method to detect low density, active malaria infection at delivery. This study has demonstrated that nPCR can be a useful tool to detect submicroscopic malaria infection in pregnant women at delivery when histology diagnosis is not available.

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NATIONALLY REPRESENTATIVE SURVEYS OF MALARIA DIAGNOSTIC CAPACITY IN THE PUBLIC SECTOR: FINDINGS FROM GHANA AND BENIN

Joseph Keating[1], Tim Finn[1], Luis Benavente[2], Chris Petruccelli[2], Nicole Whitehurst[2], Thomas Eisele[1], Gilbert Dery[3], Ekow Biney[4], Benjamin Fayomi[5], **Joshua O. Yukich**[1]

[1]Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States, [2]Medical Care Development International, Silver Spring, MD, United States, [3]DERMED Consult LLC, Tamale, Ghana, [4]Ghana Health Services, Accra, Ghana, [5]Insitut d'Sciences Biomedicales Appliquees (ISBA), Cotonou, Benin

In many African settings, malaria cases are treated presumptively. The absence of parasitological confirmation of malaria infection can lead to overtreatment of febrile illness with anti-malarial drugs, or the missing of other potentially fatal conditions. The development of rapid diagnostic tests for malaria (RDTs) combined with the scale up of Artemisinin Combination Therapies (ACTs) has led to increasing pressure to scale up parasitological diagnosis. In order to assess the availability, quality and accuracy of malaria diagnosis in Ghana and Benin, nationally representative health facility surveys were conducted in publicly supported health facilities in both countries. Results indicate that diagnostics are performed accurately a majority of the time when they are applied. The sensitivity and specificity of microscopy compared to expert readings was approximately 80% across all sampled facilities on the day of the survey. Furthermore, all observed RDTs in Ghana were interpreted correctly based on a surveyor's re-interpretation. These results appear significantly better than historic literature on malaria diagnosis with microscopy in many African locations. In Ghana, in the majority of cases, clinicians gave or prescribed drugs in line with test results. While this result is promising, it only reflects practice among patients where a test result was received. Many patients were diagnosed with malaria clinically (i.e. in the absence of any test results). While few of the patients with negative test results received a malaria diagnosis, only half of all fever patients were referred for a malaria test. Despite the overall appearance of the acceptance of testing and the agreement of clinical prescribing practice with laboratory results, approximately 30% of patients who received negative test results still received anti-malarial drugs.

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MAGNETIC DETECTION OF HEMOZOIN SURPASSES GOLD STANDARDS OF MALARIA DIAGNOSIS AND RIVALS SENSITIVITY OF MOLECULAR BASED METHODS

John R. Lewandowski[1], William C. Condit[1], Robert J. Deissler[1], Richard F. Bihary[1], Mark R. Lewandowski[1], Jason E. Jones[1], D'Arbra R. Blankenship[1], Melinda J. Zikursh[1], Arsene Ratsimbasoa[2], Moses J. Bockarie[1], Peter A. Zimmerman[1], Robert W. Brown[1], Brian T. Grimberg[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]National Malaria Control Programme, Antananarivo, Madagascar

Malaria parasites digest hemoglobin and in the process release cationic alpha-hematin, which is toxic to the red blood cell (RBC) and developing parasite. The developing parasite polymerizes this substance into chemically inert crystals known as hemozoin. Here we have exploited the paramagnetic properties of hemozoin to develop magneto-optical diagnosis (MOD) of malaria. When mixed with water, parasitized RBCs swell, burst open and release hemozoin into solution. Exposure of this lysate to an alternating magnetic field periodically aligns the hemozoin crystals so they block the transmission of light through the solution in proportion to *parasitemia*. When testing MOD on 291 samples from a malaria-endemic area we detected as few as 39 parasitized cells/μL from patients in less than1 minute with an overall accuracy of 93% compared to PCR based detection methods. Additionally, a subset of these patient samples were also compared to RDT (CareStart HRP2/pLDH (Pf/PAN)

COMBO) based detection methods which showed only 29% accuracy when compared to PCR results. Further studies of cultured parasites showed even lower detection of <1 parasitized cells/μL This device provides a rapid, robust, and inexpensive diagnosis of malaria which is an improvement over microscopic and RDT based diagnosis and allows for screenings on a population-based scale which is in line with the goals of global malaria elimination.

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SCALING-UP MALARIA RAPID DIAGNOSTIC TESTS AND ARTEMISININ-BASED COMBINATION THERAPY INTO INTEGRATED COMMUNITY CASE MANAGEMENT SITES: RESULTS FROM TWO REMOTE AND LOW-RESOURCE SETTINGS IN THE DEMOCRATIC REPUBLIC OF CONGO

John Otshudiema[1], Narcisse Embeke[2], Filiberto Hernandez[3], Jose Tchofa[1], Clarisse Mbo Modiri[4], François-Xavier Mwema[4]

[1]United States Agency for International Development, Kinshasa, Democratic Republic of the Congo, [2]Management Sciences for Health, Kinshasa, Democratic Republic of the Congo, [3]Centers for Disease Control and Prevention, Kinshasa, Democratic Republic of the Congo, [4]National Malaria Control Program, Kinshasa, Democratic Republic of the Congo

Integrated Case Management of Childhood Illness (iCCM) improves access to prompt, accurate diagnosis and effective treatment of malaria for populations with limited access to health facilities. In the Democratic Republic of Congo (DRC), a pilot study in 2008-2009 demonstrated the feasibility and the acceptability of integrated use of rapid diagnostic tests (RDTs) for malaria and artemisinin-based combination therapy (ACT) in remote villages by community health workers (CHWs). Scaling-up of the newly adopted strategy began in 2012, reaching currently 129 iCCM sites. This abstract reports the results of the scaling-up in two targeted sites in order to improve their implementation. Patients' forms filed by CHWs from two targeted iCCM sites in Kanda-Kanda health zone were reviewed to assess their adherence to the new malaria treatment guidelines. From July 2012 through March 2013, 644 sick children under five years were managed by CHWs, out of which 432 (67%) were complaining of fever for less than two days without signs of danger. RDTs were performed on 181 (42% of those with fever) children. The remaining uncomplicated cases were treated presumptively with ACTs. CHWs referred 12 severe cases to health facilities for proper case management. Among those tested, 169 (93%) had a positive RDT of which 166 (98%) were treated with ACTs. However, 11 of the 12 patients with negative RDTs were treated also with ACTs. Among those confirmed uncomplicated RDT-positive cases treated with ACTs, 68% were treated within 48 hours of the onset of fever. iCCM has the potential to improve access to prompt, effective management of uncomplicated malaria in remote, low resource settings but challenges remain to improve CHW use of RDTs for diagnosis and adherence to test results.

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USING OUTCOME-DRIVEN INNOVATION THEORY TO CLARIFY TARGET PRODUCT PROFILES FOR NEXT-GENERATION MALARIA DIAGNOSTICS

Kathleen Tietje, Christine Clerk, Kenneth Hawkins, Sarah McGray, Paul LaBarre

PATH, Seattle, WA, United States

Diagnostic tools used to reduce the burden of malaria in the control phase are less effective in regions undergoing programmatic reorientation toward malaria elimination. Next generation diagnostics for malaria elimination will need to be more accurate than microscopy and existing rapid diagnostic tests to detect the reservoirs of low-density, asymptomatic infections that perpetuate disease transmission. In addition, new diagnostics will need to be user friendly, field deployable, and capable of high throughput at low cost. Despite ongoing progress in several diagnostic development programs, the technical and market requirements for elimination phase diagnostics remain ambiguous, and therefore developers lack the incentive necessary to bring new technologies to market. To address the need for detailed target product profiles (TPPs) for elimination-specific diagnostics, PATH's project DIAMETER (diagnostics for malaria elimination toward eradication) team has identified a comprehensive list of outcome-based use-scenarios that are critical to the elimination context. Through a review of the literature and stakeholder interviews, we capture the essential system components, performance criteria, and market requirements that define success for malaria elimination stakeholders including health workers, global and national policymakers, and public and private health providers. Our findings will inform recommendations and TPPs to provide clear guidance ensuring the most efficient new diagnostic innovations are accelerated to market to support elimination campaigns.

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HIGHLY SENSITIVE RNA-BASED PARALLEL DETECTION OF *PLASMODIUM FALCIPARUM* AND *P. VIVAX* ASEXUAL STAGES AND GAMETOCYTES

Ingrid Felger[1], Rahel Wampfler[1], Felistas Mwingira[1], Sarah Javati[2], Leanne Robinson[2], Inoni Betuela[2], Peter Siba[2], Hans-Peter Beck[1], Ivo Mueller[3]

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

For studies requiring highly sensitive and simultaneous quantification of sexual and asexual stages of *Plasmodium falciparum* and *P. vivax*, 18S rRNA transcript-based detection saves efforts or costs. RNA-based positivity is considerably higher than other methods. For simultaneous highly sensitive quantification of both blood stages and gametocytes in an area of equally high prevalence of both *Plasmodium* species, we have compared and optimized different strategies for field and laboratory procedures in a cross sectional survey in 315 5-9 yr old children from Papua New Guinea. qRT-PCR was performed for gametocyte markers pfs25 and pvs25, *Plasmodium* species prevalence was determined by targeting both, 18S rRNA genes and transcripts. RNA-based parasite detection resulted in a *P. falciparum* positivity of 24%; of these 41% carried gametocytes. *P. vivax* positivity was 34%, with 36.4% of these carrying gametocytes. Sensitivity of DNA-based parasite detection was substantially lower with 14.1% for *P. falciparum* and 19.6% for *P. vivax*. Using the lower DNA-based prevalence of asexual stages as a denominator increased the percentage of gametocyte-positive infections to 59.1% for *P. falciparum* and 53.1% for *P. vivax*. Because of its easy measurability in host blood the prevalence of gametocyte carriage can be used to assess the effects of malaria interventions on transmission intensity. With optimized field procedures RNA-based assays were feasible in remote settings. This approach provides in parallel a highly sensitive measure for asexual stage prevalence.

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TOWARDS THE DEVELOPMENT OF SALIVA-BASED MALARIA DIAGNOSTICS: MASS SPECTROMETRY BASED IDENTIFICATION OF GAMETOCYTE PROTEINS IN HUMAN SALIVA

Dingyin Tao[1], Jonas G. King[1], Ceereena Ubaida Mohien[2], William John Moss[1], Sungano Mharakurwa[3], Isabelle Morlais[4], David R. Graham[2], Rhoel R. Dinglasan[1]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Johns Hopkins School of Medicine, Baltimore, MD, United States, [3]The Malaria Institute at Macha, Choma, Zambia, [4]Laboratoire de Recherche sur le Paludisme, Yaoundé, Cameroon

Robust and highly sensitive saliva-based malaria diagnostics, especially for asymptomatic carriage of *Plasmodium falciparum* gametocytes (the only mosquito-transmissible stage), are an important research priority towards the eradication of malaria. Here, we detail our plan to develop such

diagnostics and present data from our baseline proteomic analyses and initial field trials. We first built a comprehensive Mass Spectrometry (MS) workflow to establish updated proteome databases for (a) the human red blood cell, (b) human saliva and (c) *P. falciparum* gametocytes. Using this new baseline information, we developed an optimized MS protocol for determining the limit of sensitivity of gametocyte protein identification in human saliva by spiking extracted proteins from 20-200 gametocytes into 1 uL of healthy human saliva. However, the high abundance of salivary amylases, proline-rich proteins and statherin interfered with MS data-dependent scan mode and the identification of low abundance proteins was difficult. To overcome this limitation, we used peptide ligand library technology (PLLT) to "balance" protein concentrations, which can improve the gametocyte protein detection limit in spiked human saliva by 5 to 10 folds. In collaboration with clinicians in Cameroon, we then used our optimized protocol to analyze both blinded and unblinded saliva to identify candidate biomarkers for asexual and gametocyte stages of *Plasmodium*. Multi-Reaction Monitoring (MRM) will be used in ongoing experiments to validate these candidate biomarkers in individual human saliva samples. We anticipate that the confident, reproducible identification of gametocyte-specific proteins from saliva will compel the production of high-affinity rabbit polyclonal antibodies against the targeted proteins. These antibodies, once validated further by Western blot and Immunofluorescence assays, can then form the basis for the development of prototype gametocyte-specific saliva rapid diagnostic tests.

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USE OF MALARIA RAPID DIAGNOSTIC TEST RESULTS AMONG COMMUNITY MEDICINE DISTRIBUTORS IN RURAL UGANDAN COMMUNITIES: IMPACT ON APPROPRIATE TREATMENT

Richard Ndyomugyenyi[1], Pascal Magnussen[2], Sham Lal[3], Kristian S. Hansen[3], Clare Chandler[3], Sian E. Clarke[3]

[1]Ministry of Health, Kampala, Uganda, [2]University of Copenhagen, Copenhagen, Denmark, [3]London School of Hygiene & Tropical Medicine, London, United Kingdom

WHO recommends universal access to malaria diagnostics, and malaria rapid diagnostic test (mRDT) is the only feasible test at community level. Evidence regarding adherence to mRDT results by community medicine distributors (CMDs) and feasibility for use in community case management (CCM) remains limited. We assessed adherence to mRDT results by CMDs in rural Uganda, to provide information that could guide mRDT-based CCM to avoid overuse of artemisinin-based combination therapy (ACTs). A cluster-randomised trial was undertaken to examine the impact and cost-effectiveness of mRDT use by CMDs on the proportion of children receiving appropriate ACT treatment (consistent with parasitological status defined by microscopy on a research slide) in two areas with differing malaria transmission. In each setting, communities were randomised to one of two arms: ACT treatment following mRDT testing (intervention arm) was compared with presumptive treatment (control arm). Data on diagnosis and treatment were recorded by CMDs in treatment registers. Household follow-up interviews and focus group discussions were conducted with CMDs and caretakers of under-five children. Adherence to mRDT results by CMDs exceeded 85% in both transmission settings. In the high transmission area, only 44% of children seen by CMDs in the mRDT arm compared with 99% of patients in the presumptive arm were treated with an ACT, reducing ACT treatment by 55%. Similarly, in the low transmission area, less than 10% of children in the mRDT arm overall were treated with an ACT compared with 94 % in the presumptive arm, reducing ACT prescription by 87%. Analysis of whether this treatment was appropriate treatment (in line with microscopy on a research blood slide) is ongoing, and will be presented. In conclusion, training CMDs in use of mRDT in the context of CMM is feasible. CMDs performed well and adhered to malaria treatment guidelines thus improving rational use of ACTs.

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A CLUSTER RANDOMIZED TRIAL INTRODUCING RAPID DIAGNOSTIC TESTS INTO REGISTERED DRUG SHOPS IN UGANDA: IMPACT ON APPROPRIATE TREATMENT OF MALARIA

Anthony K. Mbonye[1], Pascal Magnussen[2], Sham Lal[3], Kristian S. Hansen[3], Bonnie Cundill[3], Clare Chandler[3], Sian E. Clarke[3]

[1]Ministry of Health, Kampala, Uganda, [2]University of Copenhagen, Copenhagen, Denmark, [3]London School of Hygiene & Tropical Medicine, London, United Kingdom

WHO recommends universal access to malaria diagnosis, encompassing all treatment providers, including the private sector. Diagnosis reduces inappropriate treatment practices, such as overdiagnosis of malaria and overtreatment with antimalarial drugs. Rapid diagnostic tests (mRDTs) may provide a simple means of confirming malaria diagnosis in drug shops. As yet, there is little evidence of the impact of diagnostic testing on antimalarial drug sales and referral practices by drug shops in Africa. A cluster-randomised trial to evaluate the impact and cost-effectiveness of using mRDTs, compared with presumptive treatment, has been conducted in registered drug shops in Mukono District, Uganda since October 2010. The trial aimed to evaluate the impact of mRDT testing on the proportion of drug shop clients who receive appropriate ACT treatment (consistent with parasitological status defined by microscopy on a research slide). A total of 65 drug shops were randomised to receive training either in use of mRDTs or presumptive diagnosis of malaria. All drug shop vendors (DSVs) were trained on the national malaria treatment guidelines, use of rectal artesunate pre-referral treatment, and when to refer. Supporting interventions included activities to raise community awareness emphasising that not all fevers are malaria and to test blood before receiving or purchasing an ACT. DSVs received close support supervision for first 2 months of implementation. Introduction of mRDTs in drug shops was acceptable to DSVs, the community and health staff. Adherence to mRDT results by DSVs was high with 92% of treatment decisions being consistent with mRDT test results, reducing sales of ACTs by approximately 40%, compared to drug shops in the control arm (presumptive diagnosis). Overall, appropriate treatment in drug shops using mRDTs was significantly higher than in drugs shops using presumptive diagnosis (70.1% versus 33.5%, P=0.0001). In conclusion, introducing mRDTs in drug shops was feasible and acceptable; and had a substantial impact on appropriate treatment of malaria.

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BURDEN OF MALARIA IN HIV POSITIVE PERSONS IN A MALARIA ENDEMIC AREA

Catherine O. Falade, Bukola Adesina-Adewole, Olusegun Ademowo, Isaac Folorunso Adewole

University of Ibadan, Ibadan, Nigeria

In endemic areas, malaria is usually diagnosed presumptively despite the WHO recommendation that malaria diagnosis be parasite based. HIV increases susceptibility to malaria with the result that HIV +ve persons are treated for presumed malaria very frequently. In a cross-sectional study, 2082 people living with HIV (PLWHIV) were evaluated for presence of malaria parasite by expert malaria microscopy of Giemsa stained thick blood film over a one year period. Study population was drawn from the HARVARD partnered President's Emergency Plan for AIDS Relief (PEPFAR) funded APIN adult ARV outpatient clinic, University College Hospital, Ibadan in south-western Nigeria were malaria transmission is intense. The mean age of enrolees was 36.7 ± 9.1 years (range 16-70). 81.5% (1696/2082) were female. The prevalence of malaria *parasitemia* was 15.8% (329/2082). Female PLWHIV was significantly more likely to be parasitemic than her male counterpart (13.9% versus 1.9% p<0.0001). The higher the level of education the less likely it is for patent *parasitemia*. Almost half (49.2%; 1024/2082) of the study population had one symptom or another at enrolment. The 5 most common symptoms were

Fever (70.1%), headache (63.2%), loss of appetite (44.3%), abdominal pains (32.1%), chills and rigors (22.3%) and vomiting (22.3%). Vomiting was the only symptom significantly associated with patent *parasitemia*. Temperature >37.4^{O}C was not significantly associated with malaria *parasitemia*. 43.9% had received antimalarial drugs in the preceding three months. 251 (12.1%0 reported three or more attacks of presumptively diagnosed and treated malaria in the same time frame. Thirty five (35/251; 13.9%) of these claimed to have had 6 to 10 episodes each. Drug use history in the two weeks before enrolment include antibacterial agent (15.6%), antimalarial drugs (34%) with 9% haven taken chloroquine, 12% had ACT and 12.2%sulfadoxine-pyrimethamine. 316 (15.2%) of the PLWHIV believed that they had malaria more frequently and each attack was more severe before their HIV status changed. In conclusion, malaria *parasitemia* is less frequent than earlier believed and parasite - based diagnosis will reduce over treatment with antimalarial drugs.

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RAPID DIAGNOSTIC TEST (RDT) PERFORMANCE OF THE MALARIA GOLD MINING PROGRAM IN SURINAME: COMPARING THE PERFORMANCE OF TWO RDT'S

Deborah Stijnberg, Hedley Cairo

Ministry of Health, Paramaribo, Suriname

Good Rapid Diagnostic Test (RDT) performance is at the cornerstone of the malaria diagnoses in Suriname. This because malaria infections occur mainly among persons (ca. 15,000) engaged in small-scale gold mining and related activities. Because this mining areas are remote diagnoses is primarily done by RDT either in the goldmines or in the city, in the gold miners' neighborhood, at the Tourtonne laboratory where testing occurs. End of 2011 the RDT test used switch from Binax to the use of Care tart. To assess the difference in performance of these tests from both test 82 RDT results were compared with microscopical examination by Tourtonne Laboratory (TL). Data was collected from may 2012 till April 2013. The 82 Binax results, compared with microscopy, gave a sensitivity of 76.5% compared to 94.19% for CareStart. The specificity was for both Care Start and Binax 96.9%. A PPV of respectively 86.7% and 88.9% was calculated for Binax and Care Start. Not wanting to miss positive cases the false negative rate found was 6.3% for Binax compared to 1.6% for Care Start with plasmodium *vivax* being the species being missed in both cases. Looking at the performance of the CareStart RDT test in regards to the sensitivity, specificity, PPV and the false negative rate, the Care Start has proven in the Surinamese setting to give a much better performance than the Binax. In this regard the switch from the use of Binax to Care Start was a justified and wise choice.

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NEW PULSE ASSAYS THAT MIMIC *IN VIVO* EXPOSURE REVEAL DIFFERENCES IN SENSITIVITY OF *PLASMODIUM FALCIPARUM* TO ARTEMISININ

Stanley C. Xie, Nectarios Klonis, Con Dogovski, James McCaw, Maria P. Crespo-Ortiz, Sophie Zaloumis, Julie A. Simpson, Leann Tilley

University of Melbourne, Melbourne, Australia

Artemisinins (ARTs) are the most effective class of antimalarials against *Plasmodium falciparum*. However, ART resistance has emerged in regions along the Cambodia-Thailand border and many patients in that area experience delayed clearance of blood parasites. Frustratingly, the parasites isolated from those patients do not always show reduced ART sensitivity in standard 3-day *in vitro* assays. Since ART antimalarials have short *in vivo* half-lives of 1 to 2 hours, we have applied short drug pulses to parasites in an effort to better mimic *in vivo* conditions. We hypothesised that short drug pulses may reveal stage and strain-dependent differences in drug sensitivity that are not apparent in standard 3-day assays. We have examined and compared the two laboratory strains D10 and 7G8. In standard 3-day assays, D10 has 2-3 fold higher ART IC_{50} values than 7G8 parasites. In pulse assays, tightly synchronised parasites were subjected to 4 h drug pulses at different stages throughout the intraerythrocytic asexual life cycle. Parasite viability following drug exposure was monitored in the cycle following the drug pulse by flow cytometry using the nucleotide-binding dye, Syto61. Under these conditions, the 7G8 strain exhibited up to 100 fold higher ART sensitivity than D10 parasites. Furthermore, parasites treated with 4 h drug pulses showed significant stage-dependent differences in drug sensitivity. Very early rings (less than 6 h post invasion) were very sensitive to ART. Apart from this very early stage, most of ring stage parasites (mid-ring to late-ring) were relatively insensitive to ART compared to trophozoites and schizonts. We have applied pulse assays to field isolates from the Pailin region in Cambodia that exhibit similar ART IC_{50} values in standard 3-day assays. Our results show that pulse assays can reveal large differences in sensitivity of field strains to ARTs that are not evident in standard assays, which may be clinically relevant.

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STATUS OF CHLOROQUINE RESISTANT HAPLOTYPES IN *PLASMODIUM FALCIPARUM* PARASITE POPULATIONS COLLECTED IN POST-EARTHQUAKE HAITI

Lindsay C. Morton[1], Sheila Akinyi[1], Curtis Huber[1], Meredith McMorrow[1], Michelle Chang[1], David Townes[1], Jacques Boncy[2], Roland Oscar[3], Venkatachalam Udhayakumar[1], John W. Barnwell[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]National Public Health Laboratory, Ministry of Public Health and Population, Port-au-Prince, Haiti, [3]National Malaria Control Program, Ministry of Public Health and Population, Port-au-Prince, Haiti

Haiti is located on the island of Hispaniola, the last remaining Caribbean island with endemic malaria. In Haiti, chloroquine (CQ) remains the first-line treatment for malaria. Given the challenges of conducting *in vivo* drug efficacy trials in low endemic settings such as Haiti, molecular surveillance for chloroquine resistance markers in the pfcrt gene is useful to identify emergence of resistant alleles in the population. After the January 2010 earthquake, enhanced malaria surveillance was rapidly instituted to monitor for CQ resistance and contain the spread of disease. In this study, 349 bloodspots were collected from suspected malaria cases mostly in areas in and around Port-au-Prince from March through July of 2010. We investigated the CQ resistant pfcrt markers for 121 *Plasmodium falciparum* PCR-positive samples. DNA sequencing of the pfcrt gene covering codons 72-76 was performed on PCR amplified samples. Among a total of 110 samples able to be sequenced, 108 samples were wild-type (CQ sensitive, CVMNK) while only two samples were of a resistant haplotype (CVIET). To determine if these resistant parasite alleles were imported from other endemic countries we conducted a population structure analysis using seven neutral microsatellite markers. This analysis revealed that one of the CQ resistant samples had a neutral multi-locus genotype distinct from all other Haitian samples. We were unable to amplify the other resistant parasite sample for the neutral markers. Cluster analysis of neutral microsatellite data using Structure v2.3 revealed population sub-structure with at least five distinct clusters among the CQ sensitive parasites. Furthermore, genotypes of the CQ sensitive parasites were unique to Haiti when compared to the genotypes of parasites collected in Honduras, Nicaragua, and a number of South American countries. These findings suggest a nonexistent or a very low level of CQ resistant alleles in Haiti, supporting current recommendations to use CQ as first-line treatment, while emphasizing a need for continued molecular monitoring for the emergence of antimalarial resistant parasite populations.

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GENETICALLY DISSECTING THE *PLASMODIUM FALCIPARUM* CHLOROQUINE RESISTANCE TRANSPORTER: EVALUATING FUNCTION AND EVOLUTION OF ANTIMALARIAL DRUG RESISTANCE

Stanislaw J. Gabryszewski[1], Andrew H. Lee[1], Satish K. Dhingra[2], J. Koji Lum[2], David A. Fidock[1]

[1]Department of Microbiology and Immunology, Columbia University College of Physicians and Surgeons, New York, NY, United States, [2]Department of Biological Sciences, Binghamton University, Binghamton, NY, United States

As few as four amino acid changes in the *Plasmodium falciparum* Chloroquine Resistance Transporter (PfCRT) are required to mediate malaria parasite resistance to chloroquine (CQ). An example is the Ecu1110 parasite (Ecuador) whose *pfcrt* allele is comprised of K76T, A220S, N326D, and I356L, the necessary determinant being K76T. However, in its evolution, *pfcrt* has accrued additional mutations likely to balance the requirements of enhanced drug resistance and overall parasite fitness. The current understanding of these mutations only depicts the binary states of CQ resistance and CQ sensitivity, however the contribution of each mutation in parasite fitness and drug susceptibility is unknown. Additionally, the order in which these mutations appeared is likely nonrandom, given the rarity of the emergence of CQ resistance. Using Zinc-Finger Nuclease (ZFN) technology as a tool for reverse genetics, we have edited *pfcrt* to recreate the possible evolutionary trajectory of the Ecu1110 *pfcrt* in its transition from CQ sensitive to CQ resistant. We have also investigated additional mutations to recreate the evolutionary path that parasites from Papua New Guinea might have taken as CQ was introduced in the 1950s, leveraging recent genotyping studies of old archived samples. In regenerating historical *pfcrt loci*, our goal is to narrow the possibilities of evolutionary trajectories *pfcrt* has taken to achieve CQ resistance in order to get a clearer understanding of the contributions of not only each mutation but also of each domain of PfCRT in both fitness and function. To further this, we are also using this technology to introduce novel mutations in the vacuolar loop of PfCRT, suspected to be involved in redox sensing, in order to interrogate its native function. Our studies provide further insight into the evolution of *pfcrt*-mediated CQ resistance in the context of fitness constraints, and suggest a role for this protein in maintaining solute homeostasis in the digestive vacuole.

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FIELD VALIDATION OF CANDIDATE MOLECULAR MARKERS OF ARTEMISININ RESISTANCE IN MYANMAR

Christian P. Larsen[1], Meera Venkatesan[1], Christopher G. Jacob[1], Matthew Adams[1], Hsu H. Mon[1], Kay Thwe Han[2], Myat Htut Nyung[2], Shannon L. Takala-Harrison[1], Jeffery J. Smith[1], Myaing M. Nyunt[3], Pascal Ringwald[4], Myat P. Kyaw[2], Christopher V. Plowe[1]

[1]Howard Hughes Medical Institute/University of Maryland School of Medicine, Baltimore, MD, United States, [2]Department of Medical Research (Lower Myanmar), Yangon, Myanmar, [3]Department of International Health, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, United States, [4]Drug Resistance and Containment Unit, Global Malaria Programme, World Health Organization, Geneva, Switzerland

The emergence and spread of artemisinin-resistant *Plasmodium falciparum* in Southeast Asia threatens malaria control efforts worldwide. Molecular markers of artemisinin resistance, which can be easily assayed at minimal cost, will be critical for directing surveillance and containment. A recent genome-wide association study using samples from Bangladesh, Thailand, and Cambodia identified two SNPs (MAL13-1718319 "MAL13" and MAL10-688956 "MAL10") strongly associated with delayed parasite clearance after treatment with artesunate. To validate the use of these target SNPs as potential markers of resistance, we analyzed dried blood spots obtained during a 2010 WHO Dihydroartemisin-piperaquine Therapeutic Efficacy Survey in Thanbyuzayat, Myanmar for the presence of artemisinin resistance-associated candidate SNPs. DNA was extracted from 68 dried blood spots collected prior to treatment and genotyped for the SNPs on MAL10 and MAL13 using pyrosequencing. Data were corrected using a standard curve to best estimate true ratios of sensitive to resistant genotypes for each sample. Preliminary results are as follows. For MAL10, of 66 samples successfully extracted, 21 (32%) samples contained parasites with the resistant allele, of which 18 samples (27%) were pure resistant and 3 (5%) contained both sensitive and resistant alleles. 45 samples (68%) had only sensitive MAL10 alleles present. All samples had the sensitive allele at MAL13, suggesting that the resistant allele is absent or present at very low levels in the population. Using *parasitemia* on day 3 as a surrogate for delayed parasite clearance, we analyzed the association of the MAL10 resistant allele with this phenotype. 38% (8/21) samples with resistant MAL10 had *parasitemia* on day 3, while 11% (5/45) of sensitive samples had day 3 *parasitemia*. Logistic regression indicated that the resistant MAL10 genotype was a significant predictor of *parasitemia* on day 3 (Odds ratio 4.92, p=0.015). When adjusting for log-transformed day 0 *parasitemia* in the model, the MAL10 resistant SNP became a marginally significant predictor of day 3 *parasitemia* (Odds ratio 3.90, p=0.08). MAL10 may be a valuable marker of delayed parasite clearance and should be investigated further to validate its predictive capability.

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HIGH PREVALENCE OF DHFR AND DHPS RESISTANCE HAPLOTYPES FIVE YEARS AFTER REMOVAL OF SULFADOXINE-PYRIMETHAMINE AS THE FIRST-LINE TREATMENT FOR UNCOMPLICATED MALARIA IN MALAWI

Elena M. Artimovich[1], Miriam K. Laufer[2], Kristan Schneider[3], Terrie E. Taylor[4], James G. Kublin[5], Fraction K. Dzinjalamala[6], Ananias A. Escalante[7], Shannon Takala-Harrison[1], Christopher V. Plowe[1]

[1]HHMI/Center for Vaccine Development, University of Maryland, Baltimore, MD, United States, [2]University of Maryland, Baltimore, MD, United States, [3]Department of MPI, University of Mittweida, Mittweida, Germany, [4]University of Blantyre Malaria Project, University of Malawi College of Medicine, Blantyre, Malawi, [5]Fred Hutchinson Cancer Research Center, Seattle, WA, United States, [6]Department of Pharmacy, University of Malawi College of Medicine, Blantyre, Malawi, [7]School of Life Sciences, Arizona State University, Tempe, AZ, United States

Less than a decade after the replacement of chloroquine with sulfadoxine-pyrimethamine (SP) as the first-line treatment for uncomplicated *Plasmodium falciparum* malaria in Malawi, chloroquine-sensitive parasites re-expanded in the population, to the point of renewed chloroquine clinical efficacy. Decreasing clinical efficacy due to spreading resistance mutations in dihydrofolate reductase (dhfr) and dihydropteroate synthase (dhps) caused SP to be replaced by an artemisinin-based combination therapy in 2007. Whether SP resistance in Malawi will decline in the absence of drug pressure remains unknown. Here, we report the maintenance of a high prevalence of SP resistant haplotypes five years after the removal of SP as the first-line treatment of uncomplicated malaria in Malawi. Resistance *loci* at dhfr codons 51, 59, 108, and dhps codons 437, 540, 581 were genotyped from 689 infections from 1999-2001 and 893 infections from 2012. Haplotype prevalence was estimated for both time points. SP-sensitive parasite haplotypes were not found at either time point. The prevalence of dhfr 51I/59R/108N triple mutants and dhfr 51I/108N double mutants did not change significantly between time points (85%-88%, p=0.29 and 4%-7%, p=0.06, respectively), although a decrease in dhfr 59R/108N double mutants did occur (38%-0%, p<0.001). An increase in the prevalence of dhps 437G/540E double mutants (84%-96%, p<0.001) and dhps 437G/540E/581G (0%-4%, p<0.001) was observed. The prevalence of dhps A437/K540 SP-sensitive parasites decreased from 7%-1% (p<0.001). These results suggest that although some SP-resistant haplotypes did decrease in prevalence the removal of SP as the first-line treatment of uncomplicated malaria was not sufficient to effect a return of SP-sensitive parasites. Possible explanations for these

findings include minimal fitness cost of resistant haplotypes in the absence of strong SP-drug pressure, sustained selection by the prophylactic use of SP in pregnancy and trimethoprim-sulfamethoxazole in HIV+ individuals, and/or the fixation of dhfr 108N.

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DIHYDROPTEROATE SYNTHASE 581 MUTATION IS ASSOCIATED WITH PARASITEMIA AT DELIVERY IN WOMEN WHO RECEIVED INTERMITTENT PREVENTIVE TREATMENT WITH SULFADOXINE-PYRIMETHAMINE

Julie Gutman[1], Zhiyong Zhou[1], Dyson Mwandama[2], Ryan E. Wiegand[1], Doreen Ali[3], Don P. Mathanga[4], Jacek Skarbinski[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Malaria Alert Center, University of Malawi College of Medicine, Blantyre, Malawi, [3]National Malaria Control Program, Lilongwe, Malawi, [4]Malaria Alert Center, University of Malawi College of Medicine and Department of Community Health, College of Medicine, Blantyre, Malawi

Intermittent preventive treatment in pregnancy (IPTp) with sulphadoxine-pyrimethamine (SP) is recommended for the control of malaria in pregnancy. Parasite resistance due to mutations in *Plasmodium falciparum* dihydrofolate reductase (*Pfdhfr*) and dihydropteroate synthase (*Pfdhps*) threatens its effectiveness. The *Pfdhps*581G mutation has been associated with increased placental inflammation among women receiving IPTp-SP. HIV-uninfected women with a singleton pregnancy were enrolled at delivery. Peripheral blood and placental samples were collected. Birth weight and gestational age (assessed by Ballard exam) were recorded. Nested polymerase chain reaction (nPCR) for 18S rRNA gene was done for detection of malaria; positive samples were sequenced to determine genotype at *dhfr* and *dhps loci*. We estimated a density of 20 parasites/µl for PCR positive, smear negative samples. PCR was positive in 91 of 710 samples. Genotype data for *dhps*581 were obtained for 81 samples. Of these, 10 were mutant (14%) and 71 were wild type (WT). With the exception of three samples that were not amplified at *dhfr*108, all mutant samples were mutated at *dhfr* codons 51, 59, 108 and *dhps* codons 437 and 540. All samples with *dhps*581G and 69% WT samples were from women who had two doses of IPTp-SP. The *dhps*581G mutation was associated with a positive smear (maternal peripheral, placental, or cord) at delivery (adjusted prevalence ratio (aPR) 3.0, 95% CI 2.0-4.4), even after adjusting for timing of last SP dose. *Pfdhps*581G was associated with increased parasite densities in both maternal peripheral (267 parasites/µl, 95% CI 67-1055 for mutant vs. 39 parasites/µl, 95%CI 28-54 for WT, p=0.0002) and placental (112 parasites/µl, 95% CI 43-290 for mutant vs. 36 parasites/µl, 95% CI 27-50 for WT, p= 0.01) samples. The presence of *dhps*581G was not associated with an increased risk of maternal anemia (aPR 0.55, 95% CI 0.21-1.44), histologically-confirmed placental malaria (aPR 0.90, 95% CI 0.59-1.38), a composite outcome of LBW, preterm delivery, or small for gestational age (aPR 1.48, 95% CI 0.73-2.98), or a significant change in mean birth weight (p=0.77). The *dhps*581G mutation, when present in addition to the quintuple dhps/dhfr mutant, is associated with increased maternal peripheral and placental parasite densities among SP recipients. Monitoring the prevalence of dhps581G is critical in areas where IPTp-SP is used.

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SELECTION OF CYTOTOXIC RESISTANCE TO A REVERSED CHLOROQUINE COMPOUND IN *PLASMODIUM FALCIPARUM*

Melissa Forbush[1], Steven Burgess[2], Daniel A. Daley[3], Marco Morelli[1], John C. Tan[4], Philip J. Rosenthal[5], David H. Peyton[6], Roland A. Cooper[1]

[1]Dominican University of California, San Rafael, CA, United States, [2]DesignMedix, Inc., Portland, OR, United States, [3]Old Dominion University, Norfolk, VA, United States, [4]University of Notre Dame, Notre Dame, IN, United States, [5]University of California San Francisco, San Francisco, CA, United States, [6]Portland State University, Portland, OR, United States

Antimalarial "reversed chloroquines" are comprised of a chloroquine-like moiety and a resistance reversal-like moiety, and show excellent potency against multi-drug resistant strains of *Plasmodium falciparum*. The dipyridyl analog DM1157 is highly potent against chloroquine-resistant parasites *in vitro* and *ex vivo*. It also showed good oral availability and was curative in 9/10 *P. berghei*-infected mice at doses equimolar to those at which chloroquine is effective. Like chloroquine, DM1157 inhibited beta-hematin formation *in vitro* and hemozoin formation in the parasite. While DM1157 may share a similar mechanism of action to chloroquine, it is not affected by the same *pfcrt* mutations that cause resistance to chloroquine, most significantly the K76T polymorphism. The potential for resistance to reversed chloroquines is unknown, thus we are attempting *in vitro* DM1157 resistance selection in the Dd2 line of *P. falciparum* using 24 hour on-off selection with incrementally increasing concentrations of the compound. Following drug removal, parasites are allowed to recover to 3% *parasitemia* before the next round of selection. After 48 rounds of selection, parasites showed a 2-3 fold increase in the DM1157 IC_{50}, and a 2 fold increase in the chloroquine IC_{50}, and negative cross-resistance with mefloquine. Continued selection has not resulted in further increases in IC_{50}s; rather a 4 fold increase in the LD_{50} emerged after 69 rounds of selection, indicating resistance to cytotoxic effects of the drug. The resistant parasites also showed a slow-growth phenotype. The results suggest that cytotoxic resistance to DM1157 comes at a cost of fitness, seen as slower rates of culture expansion. Parasite cloning and preparation for whole genome analysis is currently underway in order to identify the genetic determinants of resistance and parasite fitness.

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EVALUATION OF COARTEM TREATMENT FAILURES IN WEST AFRICA

Daouda Ndiaye[1], Ousmane A. Koita[2], Davis Nwakanma[3], Baba Dieye[1], Yaya D. Ndiaye[1], Lansana Sangare[2], Fatou Joof[3], Clarissa Valim[4], Sarah K. Volkman[4], Dyann F. Wirth[4], Donald J. Krogstad[5]

[1]University Cheikh Anta Diop, Dakar, Senegal, [2]University of Science, Technologies and Techniques, Bamako, Mali, [3]Medical Research Council Unit, Fajara, Gambia, [4]Harvard School of Public Health, Boston, MA, United States, [5]Tulane University Health Sciences Center, New Orleans, LA, United States

Because of concern about potential resistance (prolonged parasite clearance times) in Southeast Asia and the potential for artemisinin resistance, we have examined the effectiveness of Coartem for the treatment of uncomplicated *Plasmodium falciparum* malaria in three communities in West Africa (Gambissara in The Gambia, Dioro in Mali and Thiès in Senegal). These studies have enrolled participants 2-15 years of age with 2,000 to 199,999 asexual parasites per µl of blood who had no evidence of severe or complicated malaria and no medical problems which required treatment other than malaria. Primary endpoints for this study include asexual parasite counts <25% of baseline by day 3, clearance of all asexual parasites by day 7 and the lack of recurrent infection between days 8 and 42. Secondary endpoints include asexual parasite clearance times, ex vivo determinations of susceptibility/resistance to antimalarials such as the artemisinins and their derivatives, amodiaquine, chloroquine, quinine and pyrimethamine; testing for drug resistance markers and for presumptively

neutral markers (barcode assays). These studies have now enrolled 171 subjects with uncomplicated *P. falciparum* malaria, who have been treated with Coartem and followed for recurrent infection or other evidence of treatment failure. Of the 171 subjects enrolled, 8 have been lost to follow-up and 13 have developed recurrent infection between days 8 and 42, although there have been no early treatment failures (on or before day 7). Twelve of 13 subjects with recurrent infections had parasites at the time of recurrence with different genetic markers. The thirteenth subject had parasites with similar markers at the times of diagnosis and recurrence and delayed parasite clearance on day 3. That patient was therefore classified as having antimalarial resistance. Apart from that subject, the results obtained thus far provide no evidence for artemisinin or Coartem resistance at the community level in The Gambia, Mali or Senegal.

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SELECTION OF *PLASMODIUM FALCIPARUM* RESISTANCE TO ANTIMALARIAL ACRIDONES

Stephanie J. Huezo[1], Daniel A. Daley[2], Stephanie A. Rasmussen[1], Yuexin Li[3], Rosie Dodean[3], John C. Tan[4], Michael K. Riscoe[5], Jane X. Kelly[3], Roland A. Cooper[1]

[1]Dominican University of California, San Rafael, CA, United States, [2]Old Dominion University, Norfolk, VA, United States, [3]Portland State University, Portland, OR, United States, [4]University of Notre Dame, Notre Dame, IN, United States, [5]Veterans Affairs Medical Center, Portland, OR, United States

The need for potent antimalarials to prevent the emergence of drug resistant *Plasmodium falciparum* is urgent. Discovery of novel acridone chemotypes has shown promise for a new antimalarial drug treatment. Dual-function acridones (chemotype II) are N10 substituted, which targets the molecule to the parasite digestive vacuole where they inhibit hemozoin formation and synergize potency of other antimalarials such as quinine and piperaquine. However, the molecular target(s) of broad-spectrum (chemotype I) acridones with efficacy against both liver and blood stage malaria are unknown. Therefore, selection of acridone resistance may lead to identification of a molecular target and the mechanism of action. Using the Dd2 line and the chemotype I compound, T13, we selected stable acridone resistance by using multiple rounds of incremental, 24 hour on-off selection, followed by continuous pressure up to three times the IC_{50} value. Parasites not exposed to the initial on-off pressure have failed to develop resistance while under continuous T13 selection, thus far. A similar strategy with the chemotype II acridone, T16.5, has failed to produce resistance. Control parasites showed an T13 IC_{50} value of ~18 nM, while clonal resistant parasite lines showed an average IC_{50} of 670 nM. Cross-resistance was seen with other chemotype I acridones, but not chemotype II acridones, indicating the importance of the N10 substitution in avoiding the resistance mechanism. Only slight cross-resistance to atovaquone was seen in T13-resistant parasites. This suggests that T13 may target a unique component of the mitochondrial electron transport chain from atovaquone, which specifically inhibits ubiquinone binding to the Q_o site of the cytochrome *bc*1 complex. T13-resistant parasites are being prepared for whole genome sequencing to identify the target molecule(s) of acridone resistance.

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ARTEMETHER-LUMEFANTRINE, ARTESUNATE+AMODIAQUINE AND DIHYDROARTEMISININ-PIPERAQUINE FOR TREATING UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN UNDER-FIVE NIGERIAN CHILDREN: A RANDOMIZED CONTROLLED TRIAL

Martin M. Meremikwu[1], Friday Odey[1], Sarah Donegan[2], Chioma Oringanje[3], Angela Oyo-Ita[4], Iwasam Elemi[4], Vivian Asiegbu[3], Emmanuel Ezedinachi[3], Paul Garner[2], Umberto D'Alessandro[5]

[1]Department of Paediatrics, University of Calabar Teaching Hospital, Calabar, Nigeria, [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [3]Institute of Tropical Diseases Research and Prevention, University of Calabar Teaching Hospital, Calabar, Nigeria, [4]Department of Community Medicine, University of Calabar Teaching Hospital, Calabar, Nigeria, [5]Institute of Tropical Medicine, Antwerp, Belgium

Plasmodium falciparum is responsible for over 90% of malaria infections in Nigeria, and accounts for 30% of under-five deaths. Uncomplicated malaria in under-five children could rapidly deteriorate to severe fatal malaria if treatment is delayed or ineffective. This paper reports the findings from Afokang, one of two Nigerian sites in a multi-centre African study of the efficacy and safety of three artemisinin-based combination treatment regimens in under-five children. Trial design was open-label, parallel group randomized controlled trial. Children aged 6-59 months with uncomplicated malaria that fulfilled eligibility criteria were randomized to receive arthemether lumefantrine (AL), artesunate + amodiaquine (ASAQ) or dihydroartemisinin-piperaquine (DHAPQ). Participants were actively followed up for 28 days and then passively for 6 months. PCR was performed to distinguish recrudescent parasitaemia from new infections. Intention to treat and per protocol analysis were performed for primary outcomes assessed by D28. A total of 92, 92 and 77 eligible children were randomized to AL, ASAQ and DHAPQ groups respectively. The unadjusted D28 cure rates for AL, ASAQ and DHAPQ were 96.6% (84/87), 94.0% (78/83) and 93.1% (67/72) respectively; with PCR-adjusted D28 cure of 97.7% (84/86) for AL, and 100% for both ASAQ (80/80) and DHAPQ (70/70)..Unadjusted D63 cure rates for AL, ASAQ and DHAPQ were 93.1% (81/87), 92.8% (77/83) and 93.1% (67/72) respectively; with PCR-adjusted D63 cure of 98.8% (83/84) for AL and 100% for both ASAQ (80/80) and DHAPQ (70/70). The PCR-adjusted D28 cure rate of DHAPQ was not statistically significantly different from those of AL (OR: 0.24; 95% CI 0.00,13.25) and ASAQ (OR: 1.14 ; 95% CI 0.01,200.69). As these cure rates exceed 95%, all drugs tested meet the WHO criteria for an effective ACT. Serious adverse events were few (n=4); all four in the ASAQ group but not related to drug effects. These results confirm the appropriateness of continued use of AL and ASAQ in this locality, and have programmatic implications for wider use of DHAPQ in the country.

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CHARACTERIZATION OF THE BC1 QI SITE AS A NOVEL ANTIMALARIAL TARGET

Allison M. Stickles[1], Joanne Morrisey[2], Mariana Justino de Almeida[3], Aaron Nilsen[1], Michael Mather[2], Akhil Vaidya[2], David A. Fidock[3], Dennis E. Kyle[4], Jeremy Burrows[5], Michael K. Riscoe[1]

[1]Oregon Health and Science University, Portland, OR, United States, [2]Drexel University College of Medicine, Department of Microbiology and Immunology, Philadelphia, PA, United States, [3]Columbia University Medical Center, Department of Microbiology and Immunology, New York, NY, United States, [4]University of South Florida, Tampa, FL, United States, [5]Medicines for Malaria Venture, Geneva, Switzerland

Malaria is a tropical disease that exerts a staggering impact on health and economic productivity, due in part to the emergence of *Plasmodium* drug resistance. To counter the spread of drug resistance, the identification of novel antimalarial targets, especially those that are vital and conserved throughout the *Plasmodium* life cycle, has become a major focus of drug

development. Here, we introduce a subset of endochin-like quinolones (ELQs) that appear to inhibit the reductive (Q_i) site of the mitochondrial cytochrome bc_1 complex. The Q_i site represents a previously unreported antimalarial target that is unaffected by atovaquone resistance mutations at the *bc1* oxidative (Q_o) site, and is compatible with high potency, broad-stage antimalarial activity. Preclinical candidate ELQ-300 was used to generate resistant parasites under incremental drug pressure. Isolated clones were 10-20 fold less sensitive to ELQ-300, and contained a point mutation in the mitochondrially encoded cytochrome *b* gene. This mutation (resulting in Ile to Leu change at position 22) maps close to the cytochrome bc_1 Q_i site and has not been observed in any other malaria parasite resistant to cytochrome bc_1 complex inhibitors. Screens against the ELQ-300 resistant "D1" clone were used to identify additional potentially Q_i-selective ELQs and to pinpoint chemical features that contribute to Q_i targeting. The strongest evidence for Q_i site activity was found for ELQs with bulky chemical groups at the 6-position. Many of these sterically hindered ELQs were 100-1000 fold less potent against the D1 clone. Conversely, D1 cross-resistance was completely absent in ELQs containing small 6-position groups such as fluorine or hydrogen. ELQs containing these smaller groups retained full potency against both ELQ-300 and atovaquone resistant parasites, suggesting that a subset of ELQs are capable of circumventing ELQ-300 resistance at the Q_i site. These results provide compelling evidence that subtle structural features of the *bc1* complex influence targeting and selectivity by quinolones.

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

Aliou Traore, Demba Dembele, Bakary Sidibe, Sekou Toure, Sekou Koumare, Amadou Togo, Sanogo Kassim, Doumbo Ogobara, Abdoulaye Djimde

Malaria Research and Training Center, Bamako, Mali

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

1275

EVALUATION OF DIAGNOSTIC PLATFORMS FOR G6PD DEFICIENCY INCLUDING TWO QUANTITATIVE TESTS, THE FLUORESCENT SPOT TEST, A POINT-OF-CARE TEST AND A CYTOCHEMICAL STAINING-BASED ASSAY

Nicole LaRue[1], Maria Kahn[1], Michael Kalnoky[2], Brandon T. Leader[1], Pooja Bansil[1], Sarah McGray[1], Gonzalo J. Domingo[1]

[1]PATH, Seattle, WA, United States, [2]Tsuga Analytics, Seattle, WA, United States

A key barrier to achieving elimination of malaria caused by *Plasmodium vivax* infection is effective treatment. There is currently only one class of drugs, 8-aminoquinolines, which can entirely clear the parasite from a patient (radical cure). Unfortunately, patients with a common human trait, glucose-6-phosphate dehydrogenase (G6PD) deficiency, are at high risk of experiencing severe adverse side effects with this class of drugs. Point-of-care G6PD tests are needed to promote safe access to these drugs for patients with malaria. PATH is working with national malaria programs, manufacturers, and other key stakeholders to accelerate development and introduction of point-of-care G6PD tests where they are most needed. As part of this initiative, we evaluated different assays and platforms for determining the G6PD status of a patient. We compared the performance of a lateral flow-based G6PD test, a fluorescent spot test, and two quantitative tests for G6PD deficiency. We provide sensitivity and specificity performance data for these tests and highlight discordant test results. Discordant test results are discussed in the context of sequencing data. Additionally, we show the utility of a cytochemical staining-based assay for determination of G6PD status in addition to identification of females with heterozygous traits for G6PD.

1276

MASS DRUG ADMINISTRATION FOR THE CONTROL AND ELIMINATION OF *PLASMODIUM VIVAX* MALARIA: AN ECOLOGICAL STUDY FROM JIANGSU PROVINCE, CHINA

Michelle S. Hsiang[1], Jimee Hwang[2], Amy R. Tao[1], Yaobao Liu[3], Adam Bennett[4], G. Dennis Shanks[5], Jun Cao[3], S. Patrick Kachur[2], Richard G. Feachem[1], Roly Gosling[1], Qi Gao[3]

[1]University of California San Francisco, San Francisco, CA, United States, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Jiangsu Institute of Parasitic Diseases, Wuxi, China, [4]Tulane University, New Orleans, LA, United States, [5]Australian Army Institute, Brisbane, Australia

Recent progress in malaria control has caused renewed interest in mass drug administration (MDA) as a potential elimination strategy but the evidence base is limited. China has extensive experience with MDA but it is not well documented. We conducted an ecological study to describe the use of MDA for the control and elimination of *Plasmodium vivax* in Jiangsu Province and explore the impact of MDA on malaria incidence. We focused on two periods: 1973–1983 when malaria burden was high and MDA administered to entire counties, and 2000–2009, when malaria burden was low and a targeted approach was used in two counties. We collected all available data about the strategies implemented, MDA coverage, co-interventions, incidence, and adverse events. From 1973–1983, MDA with pyrimethamine and primaquine was used on a large scale, with annual peak coverage reaching almost 30 million people (50% of the population). Joinpoint analyses identified declines in annual incidence, -56.7% (95% CI -75.5 to -23.7%) from 1973–1976 and -12.4% (95% CI -24.7 to 2.0%) from 1976–1983. Population average negative binomial models identified a relationship between higher MDA coverage and lower monthly incidence from 1973–1976, IRR 0.98 (95% CI 0.97 to 1.00), while co-interventions, rainfall, and GDP were not associated. From 2000–2009, MDA using chloroquine and primaquine was targeted to villages and/or individuals residing near passively detected index cases (median 0.04% population coverage) and incidence declined (annual change -43.7 to -14.0%). Safety data were not collected systematically but there were rare reports of

serious but non-fatal events. In Jiangsu Province, China, large scale MDA was associated with declines in high *P. vivax* malaria transmission and a targeted approach likely contributed to interruption of transmission. MDA should be considered a key strategy for malaria control and elimination.

1277

A SAFETY MONITORING TOOL FOR PRIMAQUINE USE TO REDUCE TRANSMISSION OF *PLASMODIUM FALCIPARUM*

Eugenie Poirot[1], Jimee Hwang[2], Joelle Brown[1], Roly Gosling[1]

[1]Global Health Group, University of California, San Francisco, CA, United States, [2]Malaria Branch, Centers for Disease Control and Prevention, Atlanta, GA, United States

In 2012, the World Health Organization published new guidelines recommending the addition of a lower single dose of primaquine (PQ) (0.25 mg base/kg) than previously recommended as gametocytocidal treatment for *falciparum* malaria in areas threatened by artemisinin resistance and in settings targeting elimination. However, concerns over the small but real risks of drug-related hemolysis associated with the administration of PQ, especially in glucose-6-phosphate dehydrogenase (G6PD) deficient individuals, have restricted its widespread use. In response, this study was designed to support the safe roll-out of low dose PQ through safety monitoring for the treatment of *Plasmodium falciparum* infections to reduce transmission. We developed a tool offering enhanced monitoring to evaluate the safety and tolerability of PQ use in malaria endemic settings. The tool can assist programs to either establish a passive reporting system for adverse events related to low dose PQ therapy or a protocol for enhanced monitoring that actively tracks hematologic response. Confirmed, uncomplicated *falciparum* malaria cases prescribed PQ across all age groups from public and private health facilities are included. Assuming a population prevalence of G6PD deficiency between 5-10%, programs will need to follow 250-500 PQ treated individuals in order to detect a 25% or greater reduction in hemoglobin (Hb) between enrolment and day 7 for G6PD deficient patients with a Type I error of 0.05. For programs engaged in enhanced monitoring, follow-up visits are performed on or near day 7 after enrolment. Data on patient characteristics, malaria diagnosis and treatment, Hb levels and reported adverse events through history taking and physical examination is gathered. Finger-prick blood samples are taken for Hb, to test for G6PD deficiency, and to collect a dried blood spot. All participants are instructed on identifying symptoms of commonly reported adverse events and to monitor the color of their urine. The tool will be piloted in two low transmission countries in the Asia Pacific region and southern Africa. The design of our safety monitoring tool will be presented and could prove useful for programs planning wide-scale roll-out of single low dose PQ use in routine malaria treatment.

1278

A POSSIBLE THREAT TO THE ELIMINATION: OVERVIEW OF IMPORTED MALARIA IN JIANGSU PROVINCE, P.R. CHINA

Jun Cao, Yaobao Liu, Weiming Wang, Yuanyuan Cao, Huayun Zhou, Qi Gao

Jiangsu Institute of Parasitic Diseases, Wuxi, China

The great successful progress has been achieved in P.R. China, since the malaria elimination program launched on 2010. However, there still remain some possible threats, for example, the overseas imported cases significantly increased over the past few years. In terms of a possible resurgence of the disease, a retrospective study was conducted to describe the epidemiological profile of imported malaria 2001-2011 in Jiangsu Province, where use to be a major malaria endemic area in China. Most of imported malaria cases were acquired from African countries, young male adults with the main travel purpose of exported labors were majority of population of patients. *Plasmodium falciparum* accounted for more than 80% of the infections, and a certain proportion of patients weren't received early diagnosis and proper treatment. In recent years, the significant growth of investment to Africa and the large number of exported labors caused the increase of overseas imported cases, there is possible increasing risk of re-introduction of malaria to the country from imported cases. The web-based real time disease reporting system is core for surveillance, and the important of having an efficient response mechanism to deal with imported malaria is highlighted.

1279

A TIME-SERIES ANALYSIS OF MALARIA CONTROL AND ITS EFFECTS ON PEDIATRIC BLOOD TRANSFUSIONS IN RURAL ZAMBIA

Alison B. Comfort[1], Philip Thuma[2], Janneke van Dijk[2], Sungano Mharakurwa[3], Kathryn M. Stillman[4], Payal Hathi[1], Sonali Korde[5], Allen S. Craig[6], Nancy Nachbar[4], Yann Derriennic[4], Rose Gabert[4]

[1]Abt Associates Inc., Cambridge, MA, United States, [2]Macha Research Trust, Choma, Zambia, [3]Johns Hopkins Malaria Research Institute, Macha Research Trust, Choma, Zambia, [4]Abt Associates Inc., Bethesda, MD, United States, [5]President's Malaria Initiative, Bureau of Global Health, United States Agency for International Development, Washington, DC, United States, [6]President's Malaria Initiative, Center for Global Health, Centers for Disease Control and Prevention, Lusaka, Zambia

Malaria related mortality remains a serious burden in sub-Saharan Africa, particularly among children. Blood transfusions can reduce mortality among children with severe malarial anemia. There has been little research conducted to date to measure the impact of malaria control on the use of blood transfusions in health facilities. We report findings from a time series analysis of facility and patient record data from a rural referral hospital over an eight-year period (2000-2008). We use multivariate analyses with an auto-regression-moving-average model to assess relationships between the scale-up of malaria control and pediatric blood transfusions. We also investigate the association between malaria control scale-up and the use of blood transfusions in other patient wards. Our results show that in years when malaria control was scaled up there were 21.9 fewer pediatric blood transfusions per month as compared to years when before malaria control scale-up (95% CI 8.1-35.8; $p<0.01$), a 56% reduction. Pediatric admissions for severe malarial anemia declined over the same period. In the maternity ward, there were 1.1 additional blood transfusions per month during the years of malaria scale-up (95% CI 0.1-2.1; $p<0.05$) as compared to years before malaria scale-up. This study provides important evidence that malaria control can reduce pediatric admissions for severe malarial anemia and thereby lower the use of pediatric blood transfusions. Our findings also suggest that malaria control may provide indirect benefits to non-malaria patients through greater availability of blood resources.

1280

COMMUNITY BASED MALARIA ELIMINATION EFFORTS IN SOUTHERN ZAMBIA

Anna M. Winters[1], **Zunda Chisha**[1], Daniel Bridges[1], Benjamin Winters[1], Busiku Hamainza[2], Mercie Mwanza[2], Mulakwa Kamuliwo[2], Sichitamba Wamulume[2], Duncan Earle[3], John Miller[3]

[1]Akros, Lusaka, Zambia, [2]Zambian National Malaria Control Program, Lusaka, Zambia, [3]MACEPA, Lusaka, Zambia

Progress in malaria control efforts in Zambia resulted in a drop in malaria *parasitemia* in children under five from 22% in 2006 to 16% in 2010. This success, however, is not uniformly distributed with certain areas of Zambia reporting resurgence in malaria cases while other areas, mainly Lusaka and Southern Provinces have reached sufficiently low levels of malaria transmission to warrant an in-country push towards malaria elimination. Given this level of progress, the Zambian Ministry of Health set a goal of achieving malaria elimination in at least five areas within the country by 2015. This goal warrants the establishment of a robust malaria surveillance system with a high level of sensitivity to detect malaria infections at community level. The existing passive malaria surveillance

system, which detects malaria cases at formal health facilities, has been enhanced by leveraging volunteer community health worker networks to detect hotspots of malaria transmission through follow up and screening of households in proximity of identified index cases. These enhancements have been termed "Step 3" and constitute the final stage of an innovative three-step sequence designed to measure the progress, and move towards malaria elimination. Through Step 3, over 450 community health workers from four districts of Southern Province received a 4-day refresher training in aspects of clinical presentation, testing using rapid diagnostic tests and treatment of uncomplicated malaria according to current Ministry of Health policy. Further trainings were conducted in 2013 and will substantially increase the number of community health workers and the geographical area being considered for possible malaria elimination. Through Step 3, over 20,000 RDTs have been administered. Average positivity rate during community testing has been 11.87. Reporting completeness from community health workers each month has averaged approximately 90%. Initial results show this program increases the sensitivity and timeliness of malaria surveillance such that malaria infections previously undetected by the routine passive surveillance system are now being identified and treated. Data are being monitored by district personnel for hotspot activity to guide interventions. This presentation will highlight the efforts, successes and challenges faced during the implementation of this program.

1281

MALARIA EPIDEMIC SURVEILLANCE SITES IN THE SENEGAL RIVER VALLEY, 2008-2012

Medoune Ndiop[1], Julie Thwing[2], Mame Birame Diouf[3], Moustapha Cisse[1], Sylla Thiam[4], Mady Ba[1]

[1]Senegal National Malaria Control Program, Dakar, Senegal, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]United States Agency for International Development, Dakar, Senegal, [4]AMREF, Nairobi, Kenya

As malaria transmission falls in a region, residents may not have sufficient exposure to infective bites to maintain immunity, and it may become epidemic-prone. The Senegal River Valley is epidemic-prone and experienced malaria epidemics in the 1990s. Artemisinin-based combination therapy (ACT) was introduced in 2007, and rapid diagnostic tests (RDTs) in 2008. Mass distribution of insecticide treated nets for children under 5 years took place nationwide in 2009, and universal coverage distribution in 2011. In 2007, the Senegal National Malaria Control Program (NMCP) put in place eight epidemic surveillance sites at health posts in four districts in the Senegal River Valley. Using a standard spreadsheet, sites report the number of total consultations, suspected malaria cases, patients tested, and confirmed cases of malaria. Data quality was assessed with quarterly onsite supervision. After 2008, diagnostic effort (cases tested/cases suspected) consistently surpassed 95% and was 100% annually in half the sites, with near absolute promptness and completeness. Transmission was highly seasonal, with 80% of cases occurring from August to November, with 60% in September and October. The southernmost site was found to be inconsistent with the epidemiologic profile of the others, with a mean annual incidence of symptomatic malaria of 93/1000 over the five years. In the remaining sites, mean annual incidence of symptomatic malaria from 2009-2012 was 1.7/1000; 0.2/1000 in children under 5, 0.6/1000 in pregnant women, and 2.0/1000 in the remainder of the population. Less than 10% of all consultations were suspected malaria, and RDT positivity rate among those tested was 17%. An investigation of the cause of high incidence in the southernmost site was conducted in 2010, but no epidemics occurred during the surveillance period. Given the low incidence and simultaneous scale-up of diagnostics, it was not possible to detect the impact of vector control interventions. Epidemic surveillance sites have performed well in Senegal and increased the districts' capacity in surveillance. The NMCP continues to add sites as transmission decreases, with the goal of detecting and responding to epidemics within two weeks.

1282

WHERE TO START IN ELIMINATING AN INFECTIOUS AGENT?

Thomas Smith, Melissa Penny, Nakul Chitnis

Swiss Tropical and Public Health Institute, Basel, Switzerland

Programs that have been successful in eliminating a disease from a large area have generally concluded that they should have focused their efforts earlier in the places with the highest transmission. These remained a threat after transmission was interrupted elsewhere, leading to the need to maintain potentially expensive surveillance activities in peripheral areas after the disease has been eliminated from them. We use a simple mathematical model of cost effectiveness to consider in which order to eliminate transmission in two connected zones, given that this is technically feasible, but that resource constraints allow an attack phase in only one zone at a time. We make simple sets of assumptions about receptivity, vulnerability, and costs. Irrespective of transmission level, disease burden is minimised by attacking the higher transmission site first. In low transmission areas, costs are minimised by attacking the higher transmission site first, while if both zones have initially very high transmission, costs are minimised by attacking the lower transmission site first. These results are scale-invariant (implying the units might be small patches, villages, districts, or countries), and can be generalized to any number of units. Considerations of equity and efficiency both argue against elimination strategies that concentrate resources in areas with the lowest transmission.

1283

THE ROLES OF VECTOR CONTROL IN ENABLING MALARIA ELIMINATION CAMPAIGNS IN VARYING TRANSMISSION SETTINGS

Philip A. Eckhoff[1], Thomas R. Burkot[2], Frank H. Collins[3], James Gentile[3], Neil F. Lobo[3], Benoit Raybaud[3], Tanya L. Russell[2], Edward A. Wenger[1]

[1]Intellectual Ventures, Bellevue, WA, United States, [2]James Cook University, Cairns, Australia, [3]University of Notre Dame, South Bend, IN, United States

Planning malaria elimination programs requires an understanding of local transmission dynamics and intensities, the local vectors species, their ecologies and behaviors. Computational models of malaria transmission can then be used to simulate the effects of different combinations, timings, and durations of vector control interventions. The EMOD model was used to simulate transmission dynamics for sites in Nigeria, Kenya, Tanzania, Zambia, and the Solomon Islands where a wide range of transmission intensities are exhibited that vary by vector species with different behaviors and population dynamics. Interventions tested in silico included insecticide-treated nets, indoor residual spraying, long-lasting larvicides, spatial repellents, and attractive toxic sugar baits. The impact of timing and duration of spray and larvicide rounds were examined for impact on the human parasite reservoir during the dry season, which affected the ability of dry season drug distribution rounds to eliminate transmission. The impact of vector control interventions depended on both the baseline transmission intensity and the behavior and ecology of each local vector species, with the species composition changing in simulation as interventions were applied. These computational results demonstrate the importance of field entomological data and understanding the transmission context for elimination programs.

1284

IDENTIFYING CHILDHOOD MALARIA HOTSPOTS USING MATERNAL SEROLOGICAL RESPONSES IN ENTEBBE, UGANDA, AN AREA OF HIGH MALARIA ENDEMICITY

Juliet Ndibazza[1], Chris Drakeley[2], Simon Brooker[2], Gyaviira Nkurunyingi[1], Hellen Akurut[1], Medi Kakande[1], Pascal Magnussen[3], Birgitte Vennervald[4], Alison Elliott[1]

[1]Medical Research Council/Uganda Virus Research Institute, Entebbe, Uganda, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]Institute for International Health, Immunology and Microbiology, Faculty of Health and Medical Sciences, Copenhagen University, Copenhagen, Denmark, [4]Section for Parasitology and Aquatic Diseases, Faculty of Health and Medical Sciences, Copenhagen University, Copenhagen, Denmark

Identifying populations with the highest malaria risk can be a valuable preliminary stage in directing targeted malaria control and elimination programmes. Improving malaria surveillance in regions where malaria burden is greatest is undoubtedly essential. We hypothesised that serological markers in pregnancy can be used to identify spatial variation in childhood malaria transmission in highly endemic regions. In a randomised trial on anthelminthic use in pregnancy [ISRCTN32849447] 2,507 women were enrolled between April 2003 and November 2005, and 2,345 live births accumulated. Participants' addresses were geo-referenced using a handheld global position system (GPS). Maternal blood was collected at delivery and an enzyme immunoassay (EIA) was used to detect total IgG antibody concentrations (µg/ml) to Apical Membrane Antigen-1 (AMA-1) and Merozoite Surface Protein-1 (MSP-1). Childhood malaria episodes from birth to two years were recorded prospectively, and annual blood samples examined for asymptomatic parasitaemia. Hotspots of malaria transmission were identified by determining spatial patterns in the incidence of childhood malaria (Incidence rate/100pys (95% CI) IR=47.4, (45.3-49.5)), the prevalence of childhood asymptomatic parasitaemia determined by microscopy (10.4%, 95% CI: 8.2-12.6), and maternal levels of AMA-1 (mean $\log_{10}$=6.26, 95% CI: 6.19-6.34) and MSP-1 (mean $\log_{10}$=6.65, 95% CI: 6.58-6.71) antibodies, respectively. Two consistent hotspots were identified, and hotspots of maternal antimalarial responses to AMA-1 and MSP-1 overlapped hotspots of childhood clinical and asymptomatic malaria. Serological markers in pregnancy might be useful in identifying spatial variation in childhood malaria transmission at micro-geographic levels in highly endemic regions. Simple descriptive mapping using routine data collected at maternal and child health units, to instantly analyse epidemiological data, could be a cost-effective operational tool to detect hotspots of malaria and support planning and implementation of control activities.

1285

A MODEL FOR DISTRIBUTION OF THE CRYOPRESERVED PFSPZ VACCINE FOR FOCAL ELIMINATION OF MALARIA

Eric R. James[1], Flavia Camponova[2], Damien Huzard[2], Naïg Chenais[2], Frederic Michoud[2], Martin Page[2], Klaus Schonenberger[2], Stephen L. Hoffman[1], Marcel Tanner[3]

[1]Sanaria, Rockville, MD, United States, [2]EssentialMed, Lausanne, Switzerland, [3]Swiss Tropical and Public Health Institute, Basel, Switzerland

The PfSPZ Vaccine targeting *Plasmodium falciparum* (Pf) comprises attenuated, cryopreserved sporozoites that are stored and distributed through a liquid nitrogen (LN2) vapor phase (LNVP) cold chain using LNVP dry shippers. The vaccine is currently being evaluated in clinical trials in the U.S., Europe, and Africa. LN2 or LNVP cold chains are in common use in veterinary medicine for vaccines and artificial insemination. In human medicine LNVP storage is common, but LNVP cold chains are used on a smaller scale, principally for *in vitro* fertilization, regenerative medicine and anti-cancer vaccines. There are multiple advantages to using a LNVP cold chain, including independence from electricity. We recently reported on modeling the use of the LNVP cold chain for distribution of the PfSPZ Vaccine for use in the Expanded Program for Immunization (EPI). Using Tanzania as the example, the cost of distributing this vaccine was determined to be no different from that of distributing any newly introduced vaccine through the EPI. However, we are now aiming for use of the PfSPZ Vaccine in mass-administration to all age groups in campaigns targeting elimination of Pf malaria. For countrywide coverage, a new distribution model that incorporates a rolling series of focal campaigns based on zones, each of which utilizes a zonal storage hub, and delivery directly to immunization centers, has been developed. Zonal boundaries are defined by considerations of population density, geography, infrastructure and accessibility, and each is activated in a sequence determined by economics and seasonality of malaria transmission. We have applied this model to the distribution logistics of a 3-dose regimen of PfSPZ Vaccine for Pf elimination in Tanzania: here the model comprises 9 zones, each active for 4 months. Defining components of the distribution model are the number of doses/cryovial, the holding time and capacity of the LNVP dry shippers, the volume of, and rate of production of LN2, and the LN2 production equipment. These components can be modified according to specific zonal requirements. The model for complete population coverage for Tanzania will be presented.

1286

PERFORMANCE OF A FIELD-STABLE LAMP MALARIA KIT IN THE DETECTION OF ASYMPTOMATIC CARRIERS IN ENDEMIC AREAS OF CAMBODIA, ZANZIBAR, SWAZILAND AND COLOMBIA

Iveth J. Gonzalez[1], Nimol Khim[2], Weiping Xu[3], Andrés Vallejo[4], Bryan Greenhouse[5], Spencer Polley[6], Didier Ménard[2], Berit A. Schmidt[3], Sócrates Herrera[4], Michelle Hsiang[5], Peter Chiodini[6], David Bell[1]

[1]Foundation for Innovative New Diagnostics FIND, Geneva, Switzerland, [2]Institut Pasteur du Cambodge, Phnom Penh, Cambodia, [3]Karolinska University Hospital, Stockholm, Sweden, [4]Caucaseco Scientific Research Center, Cali, Colombia, [5]University of California San Francisco, San Francisco, CA, United States, [6]Hospital for Tropical Diseases in London, London, United Kingdom

The ability to detect asymptomatic infections at a field level will be fundamental to the success of malaria elimination strategies. This requires highly sensitive screening tests close enough to the community to enable rapid treatment. Very low parasite density infections can be detected by molecular methods such as PCR; however, these techniques require considerable training and are restricted to reference laboratories. A new field-stable CE-marked diagnostic kit for malaria based on loop-mediated isothermal DNA amplification (LAMP) is now commercially available. This LAMP kit targets mitochondrial DNA of all *Plasmodium* (Pan LAMP) or of *Plasmodium falciparum* (Pf LAMP) parasites and is able to detect down to 1 parasite/µl of blood in less than 40 minutes. This assay is not only faster than PCR, but also requires minimal processing and instrumentation, and allows test reading with the naked eye. Compared to nested PCR and using samples from febrile patients, sensitivity and specificity for Pan LAMP were <97.0% and <99.2% respectively, and for Pf LAMP <93.3% and <85%, respectively. In order to evaluate the feasibility of this LAMP kit as a tool for the detection of asymptomatic malaria, dried blood spots from volunteers in endemic areas of Zanzibar, Cambodia, Swaziland and Colombia were collected. DNA extracted by Chelex-100 or Instagene reagent was used for amplification with Pan LAMP. Nested-PCR or nested-real-time-PCR were used as reference standards. In Cambodia, based on 516 samples, sensitivity and specificity of Pan LAMP were 86.4%(95%CI:76.6-92.7) and 93.3%(95%CI:90.5-95.4) respectively while on 465 samples from Zanzibar, sensitivity and specificity were 90.7%(95%CI:78.9-96.5) and 100%(95%CI:98.8-100) respectively. In Swaziland, 921 samples have been tested by LAMP and a positivity rate of 2.7% was observed. Nested-PCR results from these samples are pending. Sample collection in Colombia is ongoing and results will be available soon. Although LAMP testing was performed in reference laboratories, previous studies have demonstrated that the same performance can be

achieved by technicians without previous training, working in simple laboratory space and with basic equipment. FIND and partners are currently working on the development of a high throughput LAMP assay with a simplified sample processing method suited to large-scale screening campaigns for malaria elimination.

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GEOGRAPHIC EFFECTS ON THE DESIGN OF A TRIAL FOR INTERRUPTING THE TRANSMISSION OF MALARIA ON A SMALL ISLAND

Mariabeth Silkey[1], Thomas A. Smith[1], Tobias Homan[2], Nicolas Maire[1], Alexandra Hiscox[2], Willem Takken[2]

[1]Swiss Tropical and Public Health Institute, Basel, Switzerland, [2]Wageningen University and Research Centre, Wageningen, Netherlands

A cluster-randomized (stepped-wedge) A hierarchical stepped-wedge is implemented in the design of a trial of the use of odour-baited traps to eliminate *P. falciparum* malaria from Rusinga Island, Lake Victoria, Kenya (SolarMal trial). Each of 4062 households to receive the intervention are grouped into clusters of approximately 50 households. Groups of nine clusters are combined into meta-clusters. One randomly selected cluster within a randomly metacluster is selected to receive the intervention each week. Hierachical randomization sequences ensured that the intervention is rolled out completely within one meta-cluster before moving on to the next randomly selected meta-cluster. A stochastic model of malaria transmission incorporating first-order community effects applied to the household geography and membership was used to measure the efficacy of the intervention at each time step. Bootstrapped confidence intervals derived from several hundred model runs were used to identify those sequences which produced narrow (± 5 % width) confidence intervals until at least the last two months of the rollout, i.e., designs with the most power to distinguish differences between the intervened and the not yet intervened groups. Results were heavily influenced by the local variations in population density, a direct effect of the physical geography of the island. Of the random sequences that met these critera, additional social constraints were applied, e.g., each meta-cluster must have an equal chance of being the first meta-cluster to receive the intervention; households within a given village must all receive the intervention within six months. Ultimately, a set fifty of the most powerful designs were presented to community representatives as alternatives, and the one to be implemented was drawn by lot.

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CHARACTERISTICS OF CHILDREN WITH ASYMPTOMATIC MALARIA PARASITEMIA IN A HIGH-TRANSMISSION SETTING OF MALAWI

Laura C. Steinhardt[1], Dyson Mwandama[2], Adam Wolkon[3], Monica Shah[1], John Gimnig[1], Themba Mzilahowa[2], Ryan Wiegand[1], Don Mathanga[2], Kim A. Lindblade[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Malaria Alert Centre, Blantyre, Malawi, [3]Centers for Disease Control and Prevention, Lilongwe, Malawi

Use of molecular diagnostics such as polymerase chain reaction (PCR) has led to the recognition that the majority of prevalent malaria infections are asymptomatic, and modeling suggests they play an important role in malaria transmission. We present data on asymptomatic *parasitemia* (AP) from a cross-sectional survey of children aged 6-59 months in Malawi enrolled in a cohort study. A census of six villages found 1,667 age-eligible children, of whom 1200 (72%) met inclusion criteria and consented. Caregivers were questioned regarding the child's illness history over the previous two weeks and a finger-prick blood sample was taken for slide microscopy and PCR. In March-April 2012, 440 (37%) out of 1186 providing a blood sample had *parasitemia* by PCR. Among parasitemic children, 291/430 (68%) had not been ill in the previous two weeks; 88% were not ill at the time of blood collection; and 89% had axillary temperature <37.5 °C. Among children not ill in the past two weeks, factors related to AP in a multivariate log-binomial model, included: age (16% increased risk per year of age, p<0.0001), wealth status (45% decreased risk for those in the wealthiest quintile, p=0.0002), and sleeping under a bednet the previous night (31% decreased risk, p<0.0001). No measured characteristics of parasitemic children differed between those reporting illness and those not, except antimalarial use in the prior two weeks (28% among symptomatic and 0% among asymptomatic children). Among PCR-positive children with blood smear results (n=326), the proportion with submicroscopic *parasitemia* was 35%. Our results suggest that fever surveys would miss more than two-thirds of malaria infections among children 6-59 months, and mass screen and treat strategies using microscopy or rapid diagnostics with similar sensitivity would miss more than a third of infections. Our results suggest that mass screen and treat with more sensitive diagnostics or mass drug administration may be required to significantly reduce the parasite reservoir in areas of moderate to high malaria transmission.

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INITIAL IMPACT OF LONG LASTING INSECTICIDE TREATED MOSQUITO NETS ON MALARIA IN KARIMUI, PAPUA NEW GUINEA

Susan Paul[1], Justin Pulford[1], Ivo Mueller[2], Peter M. Siba[1], Manuel W. Hetzel[3]

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

Malaria control activities in Karimui, a remote area in the highlands region of Papua New Guinea, have historically had limited success. For example, a national malaria control program involving indoor residual spraying and mass drug administration carried out in the 1960s reduced malaria prevalence in the general population from between 5-10% to 1% across PNG, except for Karimui where the pre-implementation prevalence rate remained unchanged despite exposure to program activities. Karimui is also relatively unique in PNG terms as reliable malaria prevalence data has been collected in the region at several intervals over the past 50 years. After decades of inactivity, the national malaria control program with support from the Global Fund to fight HIV/Aids Tuberculosis and Malaria commenced costless mass distribution of long lasting insecticide treated mosquito nets (LLINs) across PNG in 2006. Drawing on the existing evidence-base, this study aims to assess the initial impact of LLINs on malaria epidemiology in Karimui relative to previous malaria control activities in the region. A household survey (HHS) was conducted alongside an annual census update round in a Karimui-based Sentinel Site in 2011. The HHS included 255 randomly selected households from across the Sentinel site. A structured questionnaire examining household level LLIN ownership and use (among other things) was completed with the head of each participating household. A blood sample was also drawn from all consenting individuals aged 6 months or older residing in each randomly selected household (n=1135). The resulting dataset was in the final stages of cleaning at the time of drafting this abstract. Analyses will be completed by July 2013 and will include LLIN coverage and utilisation rates and malaria parasitaemia prevalence in the general population. These findings will be compared and contrasted with earlier malaria epidemiological data obtained from the Karimui region.

1290

ASSESSING THE BURDEN OF MALARIA IN LARGE CITIES OF TROPICAL AFRICA - USE OF MALARIA INDICATOR SURVEYS AND DATA FROM THE MALARIA ATLAS PROJECT

Bob Pond

JSI Research & Training Institute, Inc., Boston, MA, United States

This presentation will demonstrate a simple method using data from Malaria Indicator Surveys ("MIS files") and/or data from the web site of the Malaria Atlas Project ("MAP files") to measure the prevalence of malaria *parasitemia* among children living in large cities of tropical Africa and compare this to the prevalence among children living nearby. Geo-coordinates for each survey cluster (in the case of MIS files) or research site (in the case of MAP files) were used to determine the distance from the site to the center of the city. Geo-coordinates of any site within 25 km of the city center were entered into Google Earth to obtain a satellite image of the location and determine whether it was within the boundaries of the metropolis. Data from all sites within city boundaries were pooled together and compared to data from all sites outside of city boundaries but within 100 miles of the city center. Data from the Uganda 2009 MIS showed that the prevalence of malaria *parasitemia* among children 6 - 59 months of age living in Kampala was 93% (95% C.I.: 85% - 97%) less than among children living outside the city. Data from the Nigeria 2010 MIS showed that the prevalence of malaria *parasitemia* among children living in Lagos was 95% (95% C.I.: 82% - 99%) less than among children living outside of the city. The prevalence of malaria *parasitemia* among children living in 7 large African cities in malaria endemic areas ranged from less than 1% in Dar es Salaam to 7.9% in Monrovia and was between 72% and 95% lower than in nearby sites outside of these cities. The method provides for a practical way to use existing and easily accessible data to rigorously document the substantially lower burden of malaria in various large cities of tropical Africa.

1291

THE INTERACTION BETWEEN IRON DEFICIENCY ANEMIA AND MALARIA ON ADVERSE BIRTH OUTCOMES

Freya J. Fowkes[1], Kerryn Moore[1], Freya Langham[1], Francesca Baiwog[2], Julie A. Simpson[3], Danielle Stanisic[2], Christopher King[4], Ivo Mueller[5], Peter Siba[2], Stephen Rogerson[3], James G. Beeson[1]

[1]Burnet Institute, Melbourne, Australia, [2]Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea, [3]University of Melbourne, Melbourne, Australia, [4]Case Western Reserve University, Cleveland, OH, United States, [5]Walter and Eliza Hall Institute, Melbourne, Australia

The World Health Organization recommends iron supplementation and malaria prophylaxis during pregnancy to reduce adverse birth outcomes. However, there are concerns that iron supplementation can increase the risk of malaria and iron deficiency has been shown to protect against malaria. It is currently unknown how iron deficiency anemia and malaria interact to influence birth outcomes such as low birthweight. We determined malariametric and iron deficiency parameters (ferritin, CRP, transferrin receptor) in 470 pregnant women attending antenatal clinics in a malaria-endemic region of Papua New Guinea at enrolment (mean 25 weeks gestation). Women were followed to delivery and birth outcomes including birthweight and gestational age were recorded. The prevalence of iron deficiency anemia was high in this population (70-87%) depending on iron marker and definition. Linear regression showed an inverse relationship between ferritin (a biomarker of body iron stores) and birthweight; for every two-fold increase in ferritin, mean birthweight decreased by 58g (95%CI: -98, -18; p = 0.023) i.e. the more iron replete the greater the birthweight. There was some evidence of effect modification by the presence of *Plasmodium spp.* infection whereby the relationship between ferritin and birthweight was stronger in aparasitemic women (-67; 95% CI: -120, -15; p = 0.004) compared to parasitemic women (-31; 95% CI: -93, 31; p = 0.3). There was no association between iron status and gestational age/pre-term birth. This study provides evidence that iron deficient women are giving birth to heavier babies than their iron replete counterparts in a malaria-endemic area. Results question the use of universal iron supplementation during pregnancy for the prevention of adverse birth outcomes in malaria-endemic regions. Further research is needed to understand the mechanisms behind the protective effects of iron deficiency on adverse birth outcomes.

1292

SUBMICROSOPIC *GAMETOCYTEMIA* AND MALARIA IN MALAWI: MOLECULAR IDENTIFICATION AND IMPLICATIONS FOR TRANSMISSION

Jenna E. Coalson[1], Jenny A. Walldorf[2], Matthias J. Marti[3], Regina Joice[3], Karl B. Seydel[4], Miriam D. Ismail[1], Don P. Mathanga[5], Atupele P. Kapito-Tembo[5], Terrie E. Taylor[4], Miriam K. Laufer[6], Mark L. Wilson[1]

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

Asymptomatic *Plasmodium* parasite infections occur frequently in people where malaria is endemic, and, if gametocytemic, may represent a source of "silent" transmission that is not associated with malaria disease. Microscopy is often considered insufficient to detect gametocyte infections, which occur at low densities relative to asexual parasite stages. A novel, highly sensitive and specific reverse transcription polymerase chain reaction (RT-PCR) assay was recently created that uses 5 markers to distinguish developing and mature *P. falciparum* gametocytes from asexual stages at submicroscopic densities. We evaluated this assay using human blood samples collected during October 2012 in a cross-sectional, all-ages, household-level study of the International Center of Excellence for Malaria Research in Malawi. We aimed to define potential infectious reservoirs by assessing prevalence and predictors of submicroscopic gametocyte infection in three settings representing urban/low (Blantyre), semi-rural/mountainous (Thyolo), and rural/high (Chikhwawa) transmission. Of 2,795 people who were surveyed and sampled for *Plasmodium* microscopy, additional blood from a subset of 629 people was collected into RNAprotect for RT-PCR testing. Of these, 618 had thick smear microscopy readings. Asexual stage parasites were detected by microscopy in 9.3% (16 of 173), 3.2% (7 of 218), and 13.3% (30 of 227) of samples from Blantyre, Thyolo, and Chikhwawa, respectively. Gametocytes were also detected in one person in this subset (from Blantyre). The use of this new RT-PCR assay enabled us to identify a large number of additional submicroscopic gametocyte infections, many of which were asymptomatic in this cross-sectional sample. We present data on the predictors of submicroscopic *gametocytemia* and compare these results with microscopic and molecular results for asexual parasites. A better understanding of which humans may be unrecognized sources of parasite transmission is critical in order to enhance malaria interventions, particularly in areas approaching elimination.

1293

INCIDENCE AND FACTORS ASSOCIATED WITH CLINICAL MALARIA AMONG SCHOOLCHILDREN IN A HIGH MALARIA TRANSMISSION SETTING

Joaniter I. Nankabirwa[1], Bonnie Wandera[1], Simon Brooker[2], Moses R. Kamya[1]

[1]Makarere University College of Health Sciences, Kampala, Uganda, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom

Although school aged children bare the highest burden of asymptomatic malaria infections in high transmission settings, little is known about the burden of the clinical disease in this age-group. To investigate

the incidence and factors associated with clinical malaria among schoolchildren in Tororo, Uganda, we studied 248 children aged 6-14 years and enrolled in the placebo arm of a randomized placebo controlled trial investigating the impact of intermittent preventive treatment on malaria morbidity and cognitive function. Clinical malaria was defined as *parasitemia* with either history of fever or axillary temperature of greater than or equal to 37.50C. All children were followed for one year and clinical malaria was assessed by active case detection. Of the 248 children enrolled, 243(98%) completed the one year follow up. At baseline, *parasitemia* was present in 71(32%) of the children and the Incidence of clinical malaria was 0.34 episodes/child/year after one year of follow up. Clinical malaria episodes differed significantly by age groups with a 51% (p- value=0.029) reduction in the odds of disease in the older children (11-14 years) compared to younger children. Children infected with helminths were more likely to get clinical malaria than children without infection (OR 1.6 p-value=0.034). Interestingly, no association was observed between being parasitemic at baseline and development of clinical malaria during follow up (OR 0.96 p-value 0.891).Malaria (both asymptomatic *parasitemia* and clinical episodes) is a big health problem among schoolchildren in a high transmission setting and children may benefit from interventions targeted at reducing the malaria burden in this age-group. Combining malaria interventions to the already existing helminth control interventions in schools may provide cost effective means of extending malaria control in school aged children. Finally, in resource limited settings, targeting malaria intervention to younger children may provide considerable benefit in reducing risk of malaria and missed school days.

1294

THE OBSERVED OPTIMAL TEMPERATURE FOR MALARIA TRANSMISSION AT 25˚C IS PRECIPITATION DEPENDENT

Torleif Markussen Lunde

University of Bergen, Bergen, Norway

According to the 2007 IPCC report, the distribution and magnitude of malaria will be influenced by climate change. Exactly how is still debated. Previous studies showed that the optimal temperature for malaria transmission is 25˚C. Two studies differed with respect to how they were validated. While Lunde et al based the validation on laboratory studies, Mordecai et al used field data which showed that the highest values of EIR was observed around 25˚C. Since EIR is not only dependent on temperature, among other the availability of breeding sites, there is a possibility that this observed optimum is a result of more breeding sites in areas with temperatures close to 25˚C. To investigate whether the observed R0 maximum at 25˚C is due to more breeding sites in areas with temperatures close to 25˚C we run a previously described model, OMaWa for 20 years; one simulation with no temperature perturbation, and one simulation where air and water temperatures were perturbed with 2˚C. From the simulations we calculate the monthly mean basic reproductive number, R0, and find the breeding site dependent optimal temperature for malaria transmission. We use a non-parametric local maximum smoothing to define the temperature at which malaria is most efficiently transmitted. We find that the breeding site dependent optimum temperature for malaria transmission under current climate is ~24˚C. With a two degree increase, the observable optimum temperature for malaria transmission increases by one degree C. The model suggest the optimal temperature for malaria transmission derived from field observations is dependent on the actual air and water temperatures. To understand how climate change, ignoring changes in socio-economic conditions and interventions, influence malaria transmission and other vector borne diseases, there is a need to document the life history of vectors in relation to temperature in the laboratory.

1295

SPATIO-TEMPORAL SURVEILLANCE MODELS FOR THE DETECTION OF ELEVATED MALARIA RISK IN ETHIOPIA AND ZAMBIA

Joshua O. Yukich[1], Adam Bennett[1], Yemane Berhane[2], Honelgn Nauhasseney[2], John M. Miller[3], Busiku Hamainza[4], Marie-Reine I. Rutagwera[3], Joseph Keating[1], Thomas Eisele[1]

[1]Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States, [2]Addis Continental Institute of Public Health, Addis Ababa, Ethiopia, [3]PATH Malaria Control and Evaluation Partnership in Africa (MACEPA), Lusaka, Zambia, [4]National Malaria Control Centre, Lusaka, Zambia

In many African countries malaria transmission is being effectively reduced by broadly applied malaria prevention measures. However, there remains a need to develop methods to rapidly detect and respond to short term increases in malaria transmission. Passive surveillance data transmitted by mobile phones can provide a platform for rapid data transmission from facilities to district, national and international actors. Surveillance systems operating at scale deliver large quantities of data that require improved methods of interpretation and visualization to quickly simplify content for decision-makers. Furthermore, commonly utilized epidemic detection algorithms in most instances fail to make use of the information contained in the spatial structure of the data. Recent computational advances in spatio-temporal risk modeling using Integrated Nested Laplace Approximation (INLA) allow models accounting for spatial and temporal auto-correlation to be rapidly fit and updated, transforming surveillance data into timely and useful mapped risk notifications. Using the INLA package in R, such models were fit with high predictive accuracy for a sentinel surveillance system in Ethiopia (1,528 facility-month reports over a 39 month period in 83 facilities) and on a national HMIS dataset in Zambia (22,227 facility-month reports over a 24 month period in 1,369 facilities). These models made it possible to accurately identify spatio-temporal clusters of increased risk. When excedence probabilities were set to less than one in 1,000 for Ethiopia and less than one in 10,000 for Zambia, signaling events occurred at relative risk levels of 1,2,3, and 4 in 121, 32, 13, and 4 facility-months in Ethiopia, and in 4,175, 931, 283, and 133 facility-months in Zambia, respectively. Thresholds can be varied in this framework to balance the desired sensitivity of detection and programs' operational capacity to investigate each event. Such approaches will be of high utility to decision makers by improving both the speed and sensitivity of detection of increased malaria transmission.

1296

MALARIA TRANSMISSION IN HOUSEHOLDS IN BLANTYRE, MALAWI

Jessica W. Cassin[1], Christopher G. Jacob[1], Phillip C. Thesing[1], Osward M. Nyirenda[2], Rhoda Masonga[2], Terrie E. Taylor[3], Christopher V. Plowe[1], Miriam K. Laufer[1]

[1]Malaria Group, Howard Hughes Medical Institute/Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States, [2]Blantyre Malaria Project, University of Malawi College of Medicine, Blantyre, Malawi, [3]College of Osteopathic Medicine, Michigan State University, East Lansing, MI, United States

Epidemiological methods focused on the home, such as indoor residual spraying and the use of insecticide-treated bed nets, have proven effective in reducing the burden of malaria infection. Insight into the transmission of malaria within households might offer new strategies for malaria interventions. In urban areas, where *Anopheles* mosquitoes are rarely detected, we hypothesized that infections within households would be highly related because they were due to a single exposure outside of the urban area. We examined the relatedness of malaria infections in children participating in a clinical trial to their mothers in the city of Blantyre, Malawi. Children were enrolled in the study when they had an episode of uncomplicated malaria. Their mothers were offered the opportunity to be

tested for malaria when they felt ill. Blood spots from all of the mothers' specimens were tested for malaria by real-time PCR. We analyzed 177 new episodes of malaria in mothers among 101 individuals. Infections from the mothers and the infections detected in their children were genotyped using six neutral, unlinked microsatellite markers. Unique parasite genotypes were compared between children and their mothers and the mean proportion of shared microsatellites for the mother-child pair within a household was compared to the mean proportion of microsatellites shared among each child to every mother outside of his or her household by two sample t-test. Intra-household infections were more genetically related than inter-household infections (mean shared alleles 61.7% vs. 28.2% respectively, p value <0.0001). The extent of allele sharing suggests that drug resistant and drug susceptible parasites are passed between adults and children. Infections within a household may originate from a common source or are passed between members. If an exposure during travel outside the home introduces infection into the household, bed nets or chemoprophylaxis with travel may limit spread of malaria infection in urban areas.

1297

USING INTEGRATED LAPLACE APPROXIMATIONS TO ESTIMATE MALARIA PREVALENCE

Samir Bhatt, Daniel J. Weiss, Bonnie Mappin, Peter W. Gething

University of Oxford, Oxford, United Kingdom

Malaria transmission intensity affects almost all aspects of malaria epidemiology, including community prevalence, incidence, and total malaria mortality. The most commonly measured metric of malaria transmission is the parasite rate (PR): the proportion of individuals infected at a given point in time. Previously, models built to predict PR on a global scale (Gething et al 2010) utilised Bayesian hierarchical models fitted by Markov Chain Monte Carlo (MCMC) sampling. While these models have proven to be very useful, MCMC sampling methods become intractable in terms of both convergence and computational time when used on large data sets. This limits their utility for large-scale malaria risk mapping, particularly as the number of available PR survey data continues to grow rapidly. An alternative new framework using simplified integrated nested Laplace approximations (INLA) to compute posterior marginals (Rue et al 2008) provides a powerful and flexible alternative. Here we show that the statistical performance of the two methods for spatial prediction of PR across large areas (using West Africa as an example) is comparable in terms of predictive validation statistics. We then demonstrate the use of the INLA framework to fit larger, more complex models, which could not otherwise be fit using MCMC sampling but which provide substantial improvements in model accuracy.

1298

CREATION OF CONTINENTAL-SCALE, TEMPORALLY DYNAMIC DATASETS FROM REMOTELY SENSED IMAGERY FOR USE IN DISEASE MODELING

Daniel Weiss, Samir Bhatt, Bonnie Mappin, Peter Gething

University of Oxford, Oxford, United Kingdom

The proliferation of remotely sensed data products enables improved characterization of variables known to influence vector disease ecology. However, contamination of imagery by cloud and other data quality issues means dynamic data are often aggregated to synoptic means, limiting their utility for analyzing change. We have built two dynamic data assemblies for quantifying temperature and vegetation conditions (a lagged proxy for moisture) in Africa for use in modeling malaria. A newly designed gap-filling algorithm was central to our approach as an adjustment for persistent cloud cover in equatorial regions. Our resulting datasets consist of monthly estimates for each parameter (2000-2012, 1km spatial resolution, for all of Africa) and represent a noteworthy improvement over synoptic climatic summaries (e.g., single layers such as mean annual temperature). We discuss the implications of these new datasets for analyzing patterns and causes of changing malaria prevalence through time.

1299

RECOMBINATION AND RESOLUTION: A NOTE ABOUT *PLASMODIUM VIVAX* MEROZOITE SURFACE PROTEIN-3 ALPHA AS A MOLECULAR MARKER

Benjamin L. Rice, Monica M. Acosta, M. Andreina Pacheco, Ananias A. Escalante

Arizona State University, Tempe, AZ, United States

Parasite molecular markers can provide much needed data on *Plasmodium vivax* populations, but there have been few suitable markers identified and analyzed. One marker that has been used extensively is the gene encoding merozoite surface protein-3 alpha (MSP-3 alpha), a blood-stage antigen known to be highly variable. Here, we report the results of a study using an augmented sample of complete MSP-3 alpha gene sequences (n = 48) to analyze patterns of parasite diversity at this locus and assess its utility as a genetic marker. In addition to small study populations of Venezuelan (n = 10) and Thai (n = 17) clinical isolates, we sequenced *P. vivax* strains from a diverse range of geographic locations. Evidence of frequent and variable insertion-deletion mutations and recurrent recombination between MSP-3α haplotypes in all populations complicated the inference of genetic diversity patterns and reduced the phylogenetic signal at this locus. Comparison to results from the in silico simulation of a polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) protocol commonly used found that PCR-RFLP haplotypes were not informative of a population's genetic diversity and that identical haplotypes could be produced from analogous bands. Therefore, we question the ability of the PCR-RFLP protocol to accurately recapitulate the complex patterns of MSP-3 alpha recombination and polymorphism observed from sequencing. Our data suggest that a high number of genetic differences at the MSP-3α locus may be segregating between isolates in all *P. vivax* populations. Thus, we must caution against associating MSP-3 alpha allelic diversity with *P. vivax* population diversity, as MSP-3 alpha variability was as high within both local populations studied as in the entire diverse, global sample. High diversity allows the identification and tracking of individual parasite clones through time and space, and we suggest that this may be the most informative implementation of MSP-3 alpha as a molecular marker.

1300

A COMPARISON OF THE GENETIC STRUCTURES AMONG *PLASMODIUM* POPULATIONS FROM AREAS WITH DIFFERENT TRANSMISSION INTENSITIES IN COLOMBIA

M. Andreina Pacheco[1], Andres Vallejo[2], Socrates Herrera[2], Myriam Arevalo-Herrera[2], Ananias A. Escalante[1]

[1]Arizona State University, Tempe, AZ, United States, [2]Caucaseco Scientific Research Center and Centro Internacional de Vacunas, Cali, Colombia

Malaria low-transmission areas are of great interest because of their potential for elimination attempts. Under such conditions, the precise monitoring of infections, including their spatial connectivity (gene flow), is indispensable. In 2011 Colombia reported a total of 61,636 cases, 47% less than in 2010, which may indicate a decreasing trend. About 42% of these cases were reported in four states (departamentos): Chocó, Cordoba, Nariño, and Valle del Cauca (Valle). Some representative areas like Buenaventura, (Valle) reported 979 cases in 2012, whereas Tumaco (Nariño) and Tierralta (Cordoba) reported 1,475 and 7,482 respectively. Furthermore, these areas display differences in the relative importance of *Plasmodium falciparum* (Pf) and *P. vivax* (Pv): Valle Pv 90%, Tumaco Pv 6.9%, and Tierralta Pv 92%. We hypothesized that in Pv infections, given the presence of hypnozoites and the high prevalence of subclinical infections, we should expect higher levels of recombination and multiple infections (MOI) as compared to Pf. We also hypothesized widespread clonal expansions in Pf, due to the effect of strong selection on mutations

conferring resistance in the recent past. We analyzed a total of 120 samples including both Pv and Pf parasite samples collected from these populations using a set of physically linked and unlinked microsatellite *loci*. We found that levels of heterozygosity varied geographically in both parasites. The frequency of multiple infections (MOI) ranged from 10-20% in Pv and was about 10% in Pf. The relative low level of MOI in Pv indicates that most patients cleared up their previous infections likely due to the easy access to Primaquine. We also found strong linkage disequilibrium (clonal expansions) in both species. Thus, the pattern observed in *P. vivax* is indicative of ongoing reduced levels of recombination due to the parasite demography. Overall, the local ecology appears to explain the turnover of clones/clusters in both parasites at this spatial scale. These processes need to be taken into account when studying gene flow among malaria endemic areas.

1301

MODELING FOR MALARIA CONTROL AND ELIMINATION SCENARIO PLANNING: APPLICATION OF THE EPIDEMIOLOGICAL MODELING (EMOD) MALARIA DISEASE TRANSMISSION KERNEL TO COMMUNITY-BASED INTERVENTION DELIVERY IN SOUTHERN ZAMBIA

Edward A. Wenger[1], Philip A. Eckhoff[1], Megan Littrell[2], Kafula Silumbe[3], Busiku Hamainza[4], John M. Miller[3], Richard W. Steketee[2]

[1]Intellectual Ventures Laboratory, Bellevue, WA, United States, [2]Malaria Control and Evaluation Partnership in Africa (MACEPA), PATH, Seattle, WA, United States, [3]Malaria Control and Evaluation Partnership in Africa (MACEPA), PATH, Lusaka, Zambia, [4]National Malaria Control Center, Ministry of Health, Lusaka, Zambia

In the context of ongoing mass-screen-and-treat (MSAT) campaigns in Southern Zambia, we present the results of simulations using the Epidemiological Modeling (EMOD) program for the purpose of identifying optimal intervention strategies at different levels of endemic transmission. The EMOD modeling platform provides geographically-specific, and mechanistic stochastic models of disease transmission simulations through the use of extensive and complex software modeling. Given known or assumed parameters relevant for malaria control and elimination scenario planning for Zambia, we explore the impact of seasonality on optimal campaign timing and frequency; the cost-effectiveness of different modes of distribution, e.g. mass drug administration (MDA); the role of increased distribution and utilization of vector-control measures; and the addition of drugs with enhanced gametocidal and/or prophylactic effects such as primaquine. As malaria control and elimination efforts progress, models that optimize the combination of prevention and treatment strategies for delivery at community level are important to guide a rational approaches to choice of interventions and delivery methods.

1302

FACTORS INFLUENCING URBAN MALARIA: A COMPARATIVE STUDY OF TWO COMMUNITIES IN THE ACCRA METROPOLIS IN GHANA

Ruth C. Brenyah[1], Derick Osakunor[1], Richard Dadzie Ephraim[2]

[1]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [2]University of Cape Coast, Cape Coast, Ghana

As urban centres in Ghana continue to grow, the scale and impact of urban malaria is increasing. This study was carried out to compare the prevalence of malaria in two communities and how this may be affected by knowledge, attitudes, socioeconomic status and preventive practices of residents in two communities within the Accra metropolis. Giemsa-stained thick blood films were examined for malaria parasites in 400 people (200 each from townships with high and low urban status) from May to November 2009. Questionnaires were administered to determine and evaluate demographics of the participants. All participants lived within the two catchment areas, about 20 km apart. Average malaria prevalence among participants was 8.75%. Prevalence in Kaneshie (12%: p=0.032) was however higher than that of Airport West (5.5%). Illiteracy rate (17.5%), self-medication (81.5%) and the use of coils (21.0%) as a control mechanism was higher among residents of Kaneshie than Airport West. Most of the people (40%) in Kaneshie did not use any form of malaria control method. Insecticide spray was the most preferred malaria control mechanism by the Airport West residents (60.5%). Overall knowledge about malaria, employment status, housing conditions, level of overcrowding and the cost of treatment of malaria was better in Airport West than at Kaneshie. Malaria prevalence and factors influencing its transmission differs within communities in the same urban area. It is therefore essential to develop control and prevention strategies based on the needs of specific communities.

1303

INTERACTION OF MALARIA AND HELMINTH CO-INFECTIONS IN SYMPTOMATIC AND ASYMPTOMATIC CHILDREN IN SOUTHWEST NIGERIA

George O. Ademowo[1], Ayokulehin M. Kosoko[2], Olawunmi R. Rabiu[2], Olatubosun G. Arinola[2], Catherine O. Falade[2]

[1]College of Medicine, Ibadan, Nigeria, [2]College of Medicine, University of Ibadan, Ibadan, Nigeria

Malaria and intestinal helminth infections are common tropical diseases. Little is understood about their interaction when they coexist. We investigated the effect of co-infection of helminth and *Plasmodium* infections. Asymptomatic school children (304) and febrile children (495) were recruited from selected primary schools and Adeoyo Hospital, Ibadan, Nigeria). Blood samples were used for haematocrit determination while Giemsa stained smears were used for malaria parasite screening by microscopy. Stool samples were used for helminth diagnosis done by Kato-Katz method. Among the school children, 142 (46.7%) were positive for malaria, 181 (59.5%) had helminth only (*Ascaris lumbricoides*, AL - 43.1%, *Trichuris trichiura* TT -2.3% and AL/TT - 14.1%), while 57 (18.8%) had co-infection of helminth and *Plasmodium*. Among the febrile children, 116 (23.4%) were positive for malaria, 45 (9.1%) for worms only (AL- 7.3%, TT- 0.2%, AL/TT- 1.4%, *Taenia* spp - 0.2%) while 16 (3.2%) had co-infection of malaria and helminth. Among asymptomatic children, *Plasmodium* infection was significantly reduced in helminth positive relative to helminth negative. The opposite was the case among febrile children. Anaemia was significantly higher in *Plasmodium* infection alone compared with those with helminth infection. A. lumbricoides is the most prevalent helminth. *Plasmodium* infection was negatively and positively associated with helminth infection in asymptomatic and febrile children respectively.

1304

IDENTIFICATION AND MOLECULAR DIAGNOSIS OF A TANDEM DUPLICATION OF THE *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN GENE IN MADAGASCAR

Binta Jalloh

Case Western Reserve University, Cleveland, OH, United States

Previous studies have shown Duffy-negative African individuals are resistant to *Plasmodium vivax* (Pv) infections. However, recent findings in Madagascar confirm that Pv is capable of Duffy-independent red cell invasion. Data analysis has shown that infections from each individual field isolate are comprised of multiple Pv strains. Additionally, our whole genome sequencing of numerous Pv field isolates from Madagascar has led to discovery of a tandem duplication of the parasite's Duffy binding protein (PvDBP). The overall goal of our studies is to identify factors that play a significant role in Duffy-independent *vivax* malaria. For this study samples were collected from regions of western Madagascar where we originally identified Pv infections in Duffy-negative people. All samples were first analyzed by a *Plasmodium* species PCR-based ligation detection reaction fluorescent microsphere assay (LDR-FMA) to diagnose infection by the four species causing human malaria in Madagascar. To evaluate

complexity of Pv infection we developed nested PCR assays targeting Pv apical membrane antigen 1 gene (PvAMA1) and the PvDBP duplication to verify presence of Pv in individual infections. From a total of 138 samples, 42 were LDR-FMA-positive for Pv. Of these 0 were PCR positive for nest 1 PvAMA1, and 0 for nest 1 PvDBP duplication. However, 15 were PCR positive for the nest 2 of PvAMA1, 9 for PvDBP, and 7 were positive for both PvAMA1 and PvDBP. These results suggest that detection of Pv by focus on single-copy sequence requires nested PCR. Additionally, as Pv infections are characterized by the presence of multiple strains, our results indicate that the strains carrying the PvDBP duplication were present in approximately 25% of infections. When a PvDBP duplication strain was present, it made up varying proportions of the overall infection.

1305

SUB-NATIONAL EVALUATION OF THE IMPACT OF MALARIA CONTROL PROGRAMS IN HUAMBO PROVINCE, ANGOLA

James M. Colborn[1], Ricardo Yava[2], Gabriel Ponce de Leon[1], Adeline Chan[1], Christie Hershey[3], Filomeno Fortes[4]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Ministerio da Saude, Luanda, Angola, [3]United States Agency for International Development, Washington, DC, United States, [4]Programa Nacional de Combate à Málaria, Luanda, Angola

In most sub-Saharan African countries, malaria control activities are carried out at sub-national levels. Nation-wide activities often start with small geographical areas and expand to other areas. Monitoring and measuring the impact of interventions carried out at sub-national scale is vital to scaling these programs up to the national level, and is not always possible using national-level household surveys such as the malaria indicator surveys and demographic health surveys. These surveys are typically powered to generate estimates at either national or alternatively, one level below national scale. In Angola, we used routine health facility data to measure the impact of indoor residual spraying (IRS), insecticide treated net (ITN), and case management campaigns for malaria control that were implemented in three of the 11 municipalities in the Province of Huambo, Angola. Routine health information system data showed that suspected malaria cases in all ages decreased from 160,487 in 2009 to 135,018 in 2011, while deaths in children under 5 years of age suspected to be caused by malaria decreased from 506 to 141 (72%) during the same time period in Huambo Province. Although IRS and case management training programs were solely implemented in the Municipality of Huambo, the largest decrease in suspected malaria cases and suspected malaria mortality occurred in two municipalities that received a mass ITN distribution campaign in April 2011. In these two municipalities, the peak monthly incidence of suspected malaria cases decreased from 6.2 cases per 1000 population per month to 0.35 cases per 1000 population per month from 2011 to 2012. These results show that health facility data may be useful in measuring the impact of malaria control programs, and suggest that while the interventions in case management training and IRS appear to be decreasing the burden of malaria in Huambo Province as a whole, mass ITN distribution may have had the biggest contribution to this decrease. Other factors, such as infrastructure improvements in the Province may also have contributed to reducing malaria burden.

1306

HIGH RESOLUTION MICROSATELLITE "META-*LOCI*" TO STUDY THE MICROEPIDEMIOLOGY OF *PLASMODIUM FALCIPARUM*

Alanna Schwartz[1], Jordan Kemere[2], Molly Elmer-DeWitt[1], Libing Wang[3], Philip Rosenthal[1], Grant Dorsey[1], Bryan Greenhouse[1]

[1]University of California San Francisco, San Francisco, CA, United States, [2]Baylor College of Medicine, Houston, TX, United States, [3]University of California Berkeley, Berkeley, CA, United States

Genotyping information may be used to provide fine scale data on parasite transmission networks, especially in low transmission areas. Microsatellites can be easily amplified from field samples and can readily identify alleles from multiple strains present in the blood. However, when performing many pairwise comparisons between samples, the probability of numerous alleles matching between unrelated parasites due to chance is unacceptably high, even when homozygosity at each locus is relatively low (~0.2). To improve genetic resolution, we investigated a "meta-locus" approach, in which the haplotypes of genetically linked microsatellites, instead of individual microsatellites, were used to define each locus. We identified 27 additional microsatellites demonstrating variability located within 15kb (with the exception of one due to an absence of closer microsatellites) of 10 commonly used microsatellites (range1-4 per locus), and thus unlikely to recombine over a few generations. Multiplex nested PCR methods were developed to increase sensitivity while conserving DNA. These methods were 10-100x more sensitive than individual PCR reactions, amplifying dried blood spot (DBS) samples with parasite densities of 10-100 parasites / ul. Preliminary genotyping data obtained from 50 dried blood spot samples in Uganda demonstrates that discrimination is increased on average 3.8 fold at each locus, with homozygosity decreasing from a median of 14% (interquartile range of 10%-13.5%) to 4% (interquartile range of 3.65%-4.55%). A number of meta-*loci* had unique signatures for almost all samples tested, indicating that homozygosity may have been overestimated in the samples tested. These methods offer promise for obtaining highly discriminatory multilocus genotypes from field samples. Application of these methods to evaluate fine-scale population structure is in process. In particular, samples from pre-elimination areas are being evaluated to identify the source and spread of malaria infections to better target interventions.

1307

WEALTH STATUS AND DEMAND FOR MALARIA TREATMENT FROM PRIVATE SECTOR RETAILERS IN NIGERIA

Eric Schatzkin[1], Jenny Liu[1], Naomi Beyeler[1], Sepideh Modrek[2], Anna De La Cruz[1], Dominic Montagu[1]

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

Although AMFm subsidies have increased the supply of artemisinin combination therapies to treat malaria, there is little access to reliable malaria diagnostics in Nigeria even though national policy calls for parasitological confirmation prior to treatment. In 2012, we conducted REMEDI, a pilot study of the acceptability of malaria rapid diagnostic tests (RDTs) among adult customers purchasing anti-malarials from retail pharmacy and proprietary and patent medicine vendors (PPMVs) in urban and peri-urban areas of Oyo State in Southwest Nigeria. Using the pilot study data, our analysis aims to (1) assess the representativeness of our sample compared to a national survey and (2) investigate differences in malaria-treatment seeking behavior, acceptability of RDTs, and treatment adherence by individuals of different wealth statuses. To enable external comparison, wealth indexes are constructed using principal components analysis of household assets measures collected in both REMEDI and the 2010 Malaria Indicators Survey (MIS). Indexes are then converted to quintile categories according to cutoff values defined by the MIS and used as the main predictive indicators in subsequent bivariate and multivariate regression analyses.A comparison of wealth quintiles between REMEDI and the MIS shows that the REMEDI sample is substantially wealthier than the national population and concentrated within the top two wealthier quintiles. Regression analyses indicate that individuals in the highest wealth quintile are significantly less likely to be recruited at a PPMV, but more likely to report having gone to a PPMV for the previous episode of suspected malaria. The wealthiest also paid somewhat less for their drugs. No differences in other health-seeking behaviors, types of anti-malarials purchased, or RDT-positivity was detected, but the wealthiest individuals were less likely to take the correct treatment (according to the RDT result) even though they were more likely to consult the treatment advice card given to them by the study nurse. While the wealthiest individuals were also more educated, acceptability and adherence to RDT results may be more problematic and require targeted intervention.

1308

DIFFERENCES IN THE EPIDEMIOLOGY OF MALARIA IN THE GAMBIA, SENEGAL AND MALI

Seydou O. Doumbia[1], Jean-Louis Ndiaye[2], Abdullahi Ahmad[3], Mahamadou B. Toure[4], Sory I. Diawara[4], Joseph Okebe[3], Ousmane A. Koita[4], Mahamadou Diakite[4], Lansana Sangare[4], Nafomon Sogoba[4], Joseph Keating[1], Umberto D'Alessandro[3], Donald J. Krogstad[1]

[1]Tulane University Health Sciences Center, New Orleans, LA, United States, [2]University Cheikh Anta Diop, Dakar, Senegal, [3]Medical Research Council Unit, Fajara, Gambia, [4]University of Science, Technologies and Techniques, Bamako, Mali

Wide-scale deployment of improved access and coverage with malaria control tools will reduce malaria transmission in highly endemic areas and may ultimately lead to the elimination of malaria as a major public health problem in West Africa. In order to monitor changes in malaria epidemiology related to policy, both cross-sectional and cohort study designs have been used to target representative samples of the local population in 3 West African Countries: The Gambia, Mali and Senegal. Two sites in Mali characterized by high and intense transmission (irrigated sahelian areas of Dioro and Sudan Savana areas of Dangassa) have been selected. In Senegal, the Thies site is urban with moderate seasonal transmission, whereas the Gambian site is rural and has achieved a significant reduction in the intensity of transmission. We report here the results of 2 cross-sectional surveys carried out in the rainy and dry season respectively at the Mali sites and one cross sectional survey performed in the rainy season at the sites of Gambia and Senegal. The prevalence of asymptomatic infection in all age groups included during the rainy season varies from 0.3% (4/1497) in Urban Thies, 3.4% in rural Gambissara (48/1401), 20.4% in irrigated site of Dioro (301/1479), to 42.6% (601/1412) in Sudan Savana of Dangassa. The prevalence of symptomatic malaria within 2 weeks period in the same cohort was 0% in Thies (N=1497), 1.8% in Gambissara (N=1401), 2.1% in Dioro (N=1479) and 9.4% (N=1412) in Dangassa. During the dry season (February-March) the prevalence of asymptomatic infection in the same cohort remained relatively high in Dangassa : 45.8% (N= 1153) and lower in Dioro 8.4%. These results shows the challenges in control in high endemic areas such as Dangassa. In addition to transmission patterns, the differences between these 4 sites may reflect the different levels of use and coverage with malaria intervention tools.

1309

WHO CRITERIA FOR SEVERE MALARIA IN IDENTIFYING SEVERE *VIVAX* MALARIA: PRELIMINARY DATA FROM A STUDY IN IQUITOS, PERU

Edward S. Smith-Nuñez[1], Salomon Durand[1], G. Christian Baldeviano[1], Antonio M. Quispe[1], Frederique Jacquerioz[2], Moises Sihuincha[3], Juan C. Celis[4], Lorena Tapia[1], Karen Campos[1], Kimberly A. Edgel[1], Andres G. Lescano[1]

[1]U.S. Naval Medical Research Unit - 6, Lima, Peru, [2]Tulane University, Lima, Peru, [3]Apoyo Iquitos Hospital, Lima, Peru, [4]Iquitos Regional Hospital, Lima, Peru

Vivax malaria is responsible for 90% of malaria cases in Peru. Severe *vivax* malaria is defined using the WHO criteria devised for *Plasmodium falciparum*, but may lead to misclassification in *vivax* malaria. We report preliminary findings from a case-control study for severe *vivax* malaria. The study is being conducted in Iquitos, in the Peruvian Amazon. Participants were PCR confirmed *P. vivax* mono-infection 5 to 65 years old. Cases were defined using the WHO severe malaria criteria. Controls were uncomplicated *vivax* malaria, two for each case. Criteria for critically ill malaria case included very severe anemia (hemoglobin <5 mg/dL), lung injury, shock, renal failure, admission to the ICU or cerebral malaria. All cases and controls provided informed consent and were treated by the Ministry of Health following local guidelines. Thirty cases and 59 controls were enrolled based mainly on clinical criteria. None of the subjects tested positive for dengue or leptospirosis. The main characteristic of cases was prostration (96%). Other characteristics at admission were severe anemia (n=2), seizures (n=1), coma (n=1), jaundice (n=2) and pulmonary alterations (n=3). After 24 hrs, when the laboratory results were available, 11 controls (19%) were re classified as cases due to total bilirubin > 2.5 mg/dL (n=9), glucose < 60 mg/dL, and hematocrit <21% (n=1). No subjects presented with altered renal laboratory parameters. Prostration was the only severity criteria in thirteen cases (32%). Neither of these cases met the criteria for critically ill patients as defined above. Seventeen subjects were critically ill. Prostrated-only cases, compared to controls, had no differences in hemoglobin, platelets or creatinine, but they had higher total bilirubin levels (76% vs 35%, p=0.008) and lower albumin levels (30% vs 93%, p<0.001). All but one subject were discharged from the hospital within three days. There is a need for a specific definition of severe *vivax* malaria. Prostration may be a sensitive but not specific criteria to identify severe and critically ill *vivax* cases.

1310

LOOKING FOR GOLD, FINDING MALARIA: 2012 MALARIA SURVEILLANCE IN GOLD MINERS' COMMUNITIES IN SURINAME

Hedley Cairo[1], Deborah Stijnberg[2], Marjorie Ardjosentono[1]

[1]Ministry of Health Malaria Program; "Looking for gold, finding malaria", Paramaribo, Suriname, [2]Ministry of Health, Paramaribo, Suriname

Despite the marked reduction of malaria incidence in Suriname, malaria continues to affect the migrants' population (n= 15,000) involved in gold mining. Miners have been trained in the use of RDTs and treatment of uncomplicated malaria to provide services in their communities. Blood films are prepared for the quality control of all RDTs performed. They report to the Tourtonne laboratory (TL). The TL in the epicenter of the Brazilian gold miners' community in the city is the other component of malaria surveillance in gold miners' communities. The TL staff executes Active Case Detection Campaigns on a regular basis in gold mining areas. The surveillance data serves as the basis of this paper. In 2012, 321 cases were recorded, representing a decrease of 50.3% from the 646 recorded in 2011. *Plasmodium falciparum*, *P. vivax* and *P. malariae* were identified in 42.4%, 49.5% and 1.9% of cases respectively. 3.1% had a mixed infection. For 3.1% the species could not be determined. 259 (80.7%) cases were imported; the 62 autochthonous cases signify a reduction of 62.4% compared to the 165 reported in 2011. Of the autochthonous cases, 30 (55.6%) were acquired in the Lawa basin, 11 (20.4%) around the Lake, 6 (11.1%) in the Saramacca and 3 (5.6%) in the Marowijne basin. The Upper Marowijne had the lowest number of cases 1 (1.9%). The 62 cases were dispersed over 24 locations with 5 or less cases per location. 52.4% of the locations had only 1 malaria case in 2012. The mean prevalence measured during ACDs was 1.8%. The SPR was 5.9%, ABER 36.2% and API 5.2 per 1000. 98.4% of the infections occurred in Brazilians. 4 cases were reported in pregnant women. Increased access to diagnosis and treatment in the remote gold mining areas, ACD campaigns and the distribution of LLIN to the populations at risk in the gold mining areas appears have contributed to the steep decline in malaria cases. An increasing proportion of the malaria cases appear to be acquired in French Guiana. A regional approach is mandatory to reduce cross-border importation.

1311

THE IMPACT OF MALARIA CONTROL INTERVENTIONS IN ETHIOPIA, 2000-2012

Kesetebirhan Admasu[1], Daddi Jima[2], Ashenafi Assefa[2], Hiwot Solomon[1], Asnakew Yeshiwondim[3], Zelalem Kebede[3], Gunewardena Dissanayake[4], Hiwot Teka[4], Sheleme Chibsa[4], Lia Florey[5], Cameron Taylor[5], Jimee Hwang[6], Joseph Malone[7], Christine Hershey[8], Carrie Nielsen[6], Yemane Berhane[3], Amha Kebede[2]

[1]Ethiopian Federal Ministry of Health, Addis Ababa, Ethiopia, [2]Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia, [3]Addis Continental Institute of Public Health, Addis Ababa, Ethiopia, [4]President's Malaria Initiative-United States Agency for International Development, Addis Ababa, Ethiopia, [5]ICF International, Calverton, MD, United States, [6]President's Malaria Initiative-Centers for Disease Control and Prevention, Atlanta, GA, United States, [7]President's Malaria Initiative-Centers for Disease Control and Prevention, Addis Ababa, Ethiopia, [8]President's Malaria Initiative-United States Agency for International Development, Washington, DC, United States

Malaria transmission in Ethiopia is unstable, usually limited to areas <2000m in altitude, and 57 million people are considered at risk. Periodic, widespread malaria epidemics with high mortality have been historical features. However, since 2004, Ethiopia rapidly scaled up effective malaria interventions including: artemisinin-based combination therapies (ACTs), indoor residual spraying, long lasting insecticidal nets (LLINs), and universal laboratory diagnosis by microscopy or rapid diagnostic tests (RDTs). In addition, the total number of public health facilities increased 5-fold and >34,000 additional health extension workers were trained in malaria case management and supplied with RDTs and ACTs, which significantly increased Ethiopia's outpatient and inpatient malaria care capacity. Multiple data sources including program, survey, and surveillance data were reviewed to assess the scale-up of interventions and its potential impact. Although no ACTs were available in Ethiopia prior to 2004, more than 4 million treatment doses have been distributed yearly since 2006, sufficient to treat the nation's reported *Plasmodium falciparum* cases. Laboratory confirmation of all suspected malaria cases with microscopy or RDTs increased from <25% in 2004 to 83% by 2012. According to the national Malaria Indicator Surveys, 55% of households owned at least one LLIN in 2011 and access to malaria diagnosis and treatment services within 24 hours of fever onset has increased from 15% in 2007 to 51% in 2011. These improvements coincided with a 28% decrease in mortality from 2005 to 2011 among children less than five years of age. In addition, widespread malaria outbreaks have decreased. In 2003, a large-scale malaria outbreak resulted in an estimated 25,000 malaria-related deaths among children less than five years of age affecting 211 districts. Since then, fewer than 12 districts per year have reported malaria outbreaks. The scale up of malaria control interventions, treatment of laboratory-confirmed malaria cases with ACTs, and health systems strengthening are associated with reductions in annual malaria deaths among children less than five years of age and the suppression of malaria epidemics in Ethiopia.

1312

GENETIC DIVERSITY OF *PLASMODIUM VIVAX* INFECTIONS IN A REMOTE FORESTED AREA OF CENTRAL VIETNAM

Nguyen Van Hong[1], Pham Vinh Thanh[1], Peter Van den Eede[2], Christopher Delgado[3], Nguyen Thi Huong Binh[1], Chantal Van Overmeir[2], Umberto D'alesandro[4], Duong Thanh Tran[1], Anna Rosanas-Urgell[2], Annette Erhart[2]

[1]National Institute of Malariology, Parasitology and Entomology, Hanoi, Vietnam, [2]Malariology Unit, Institute of Tropical Medicine, Antwerp, Belgium, [3]Unit of International Health, Faculty of Medicine, University of Antwerp, Antwerp, Belgium, [4]Malariology Unit, Institute of Tropical Medicine Antwerp, Belgium; Diseases Control and Elimination Theme, Medical Research Unit, Fajara, Gambia

Plasmodium vivax control is becoming increasingly important in Vietnam where the malaria burden has been drastically reduced and the government has now engaged into a malaria elimination program. Understanding *P. vivax* transmission dynamics is crucial for further improving elimination strategies; however this knowledge remains scarce in most endemic areas. We present the baseline data on the *P. vivax* population genetics in a remote area of Central Vietnam. Two hundred and forty five blood samples collected before treatment (day 0) in *P. vivax* patients were submitted to species-specific PCR for diagnosis confirmation. All *P. vivax* mono-infections were genotyped using 16 previously published microsatellites. The overall genetic diversity and structure of the *P. vivax* parasite population was determined and related changes in space, time and demographic indicators were analyzed. A total of 239 patients were confirmed to be *P. vivax* mono-infections and genotyped. Overall the *P. vivax* population displayed a high genetic diversity with an expected heterozigosity (He) of 0.70 and an average of 1.21 alleles/locus (ranging from 1 to 5 alleles/locus). Most of the infections were polyclonal (75.3%) with an average multiplicity of infection of 2.7 haplotypes/person. The risk of polyclonal infections ranged from 50% to 96% across villages, and was significantly higher in children compared to adults (73.8 % versus 26.2%). Moreover, compared to dry season, the risk of polyclonal infections was 9-fold higher during the rains. In conclusion, in this remote, forested area, the *P. vivax* population was highly diverse and polyclonal, indicating substantial ongoing transmission; interrupting it may require additional and new interventions to those currently deployed.

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SEQUENCING OF *PLASMODIUM FALCIPARUM* LIVER STAGE CD8 T CELL ANTIGENS TO IDENTIFY VACCINE CANDIDATES

Xiaoyan Zou, Sri Hadiwidjojo, Jessica Bolton, Joao Aguiar, Thomas Richie, Eileen Villasante, Vince Gerbasi

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

CD8 T cell mediated immunity is a critical arm of the immune response in radiation attenuated sporozoite (RAS) conferred protection against malaria. When parasite development is halted inside hepatocytes, malaria peptides are presented on the surface of infected hepatocytes through MHC Class I receptors for presentation to CD8 T cells. Characterizing *Plasmodium falciparum* (*Pf*) peptides presented on the surface of infected hepatocytes holds promise to identify novel liver stage antigens as vaccine candidates. Here we report that by establishing an *in vitro* system of liver stage schizont culture and utilizing state-of-the-art mass spectrometry approaches we have successfully identified *Pf* peptides expressed on human primary hepatocytes from various donors at 48- or 96-hrs after sporozoite inoculation. From these samples we were able to identify immunogenic *Pf* liver stage peptides that match several HLA supertypes from primary human hepatocytes. By continuing our screening process we will work to identify the full repertoire of *Pf* liver stage antigens as a pathway to accelerate pre-erythrocytic antigen discovery.

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PLASMODIUM ALVEOLIN 5 IS ESSENTIAL FOR THE NORMAL FORMATION OF INNER MEMBRANE COMPLEX OF OOKINETES

Naoaki Shinzawa[1], Eizo Takashima[2], Riko Katsube[1], Mamoru Nozaki[1], Mayumi Tachibana[1], Aki Kato[2], Amporn Thongkukiatkul[3], Takafumi Tsuboi[2], **Motomi Torii**[1], Tomoko Ishino[1]

[1]Proteo-Science Center, Ehime University, Toon, Ehime, Japan, [2]Proteo-Science Center, Ehime University, Matsuyama, Ehime, Japan, [3]Department of Biology, Burapha University, Chonburi, Thailand

Malaria parasites undergo multiple developmental stages and adopt a range of cell shapes, including both motile and non-motile forms. While the motility requires the motor complex associated with the plasma membrane of elongated shape, it remains an open question whether the cell shape itself is required for the differentiation to next developmental stage. All invasive stage parasites have submembranous flattened vesicle packed into continuous layer, called inner membrane complex (IMC), supporting the plasma membrane. We found that ALV5, a member of *Plasmodium* alveolin, is essential for the normal formation of IMC of ookinetes using knocking down of ALV5 in mosquito stage. ALV5-deficiency resulted in the developmental arrest at a point of apical end formation from remnant zygote and the extremely low invasion ability to mosquito midgut due to its lost motility. However, intrahemocoel injection of arrested parasites resulted in normal development of sporogonic stage. These findings clearly indicate that the molecular machinery for differentiation is developed independently of cell shape of parasites.

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RALP1, A RHOPTRY-NECK, ERYTHROCYTE-BINDING PROTEIN OF *PLASMODIUM FALCIPARUM* MEROZOITES, IS A NOVEL VACCINE CANDIDATE ANTIGEN

Daisuke Ito[1], Tomoyuki Hasegawa[1], Tsutomu Yamasaki[2], Thangavelu U. Arumugam[1], Kazutoyo Miura[3], Amporn Thongkukiatkul[4], Satoru Takeo[1], Carole A. Long[3], Jetsumon Sattabongkot[5], Eun-Taek Han[6], Eizo Takashima[1], Motomi Torii[1], Takafumi Tsuboi[1]

[1]Proteo-Science Center, Ehime University, Matsuyama, Ehime, Japan, [2]Department of Life Sciences, Faculty of Science, Okayama University of Science, Okayama, Japan, [3]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [4]Department of Biology, Faculty of Science, Burapha University, Chonburi, Thailand, [5]Mahidol Vivax Research Center, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, [6]Department of Medical Environmental Biology and Tropical Medicine, School of Medicine, Kangwon National University, Chuncheon, Republic of Korea

Erythrocyte invasion by merozoites is an obligatory stage in *Plasmodium* infection and is essential to malaria disease progression. Proteins in the apical organelles of the merozoites mediate invasion into the erythrocytes, and are potential malaria vaccine candidates. The rhoptry-associated, leucine zipper-like protein 1 (RALP1) in *P. falciparum* was previously found to be specifically expressed in schizont stages and localized to the rhoptry of merozoites based on immunofluorescence assay (IFA). Also, RALP1 has been refractory to gene knockout attempts, suggesting that it is essential for blood stage parasite survival. These characteristics suggest that RALP1 is a potential blood-stage vaccine candidate antigen, and here we aimed to assess its potential in this regard. Antibodies were raised against recombinant RALP1 proteins synthesized using the wheat germ cell-free system. Immunoelectron microscopy demonstrated for the first time that RALP1 is a rhoptry neck protein of the merozoites. Moreover, our IFA data showed that RALP1 translocates from the rhoptry neck to the moving junction during merozoite invasion. Growth and invasion inhibition assays revealed that anti-RALP1 antibodies inhibit invasion of erythrocytes by merozoites. Erythrocyte binding assays revealed that RALP1 possesses an erythrocyte binding epitope in the C-terminal region, suggesting that RALP1 represents a new *P. falciparum* erythrocyte binding protein. Human sera collected from malaria endemic areas in Thailand and Mali recognized this protein. Overall, our findings indicate that RALP1 is a rhoptry-neck erythrocyte-binding protein, and that it merits additional evaluation as a *P. falciparum* blood-stage vaccine candidate.

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NOVEL MOLECULE THAT IS SPECIFICALLY EXPRESSED ON MALE GAMETOCYTE AND MICROGAMETE HAS POTENTIAL ROLE ON EXFLAGELLATION

Mayumi Tachibana, Motomi Torii, Moe Sudo, Yuki Yokouchi, Takafumi Tsuboi, Tomoko Ishino

Proteo-Science Center, Ehime University, Matsuyama, Japan

Plasmodium transmission via mosquitoes required sexual stage parasite development and fertilization in mosquito midguts. After ingestion of gametocytes by mosquitoes, fertilization occurs to form zygotes, which develop into invasive forms, ookinetes. These transmission events occur rapidly within 24 hours after blood meals and many molecules are supposed to be involved. Nevertheless, little is known about molecular mechanisms how parasites transmit to mosquitoes. Previously, we reported a novel male specific protein, designated PyGM75, that is expressed in both gametocyte and gamete stage of *Plasmodium yoelii*. In this study, the subsellular localization of PyGM75 in male gametocytes and gametes were examined by immune-electron microscopy. It is revealed that PyGM75 is localized to the electron dense organelles, named osmiophilic bodies of male gametocytes, then transported to the surface of microgametes. Next, we produced pygm75 gene disrupted parasites to elucidate its function. Pygm75 disrupted parasites can normally differentiate into male and female gametocytes. However, these parasites were drastically impaired the exflagellation ability, therefore they could not form oocysts in the mosquito midguts. Further electron microscopic analysis demonstrated that osmiophilic bodies were disappeared from pygm75 disrupted male gametocytes. These results indicate that PyGM75 plays a crucial role in microgametes formation prior to fertilization.

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CONTRASTING ROLES FOR PFACS5 AND PFACS9 IN THE EXPANDED *PLASMODIUM FALCIPARUM* ACYL CO-A SYNTHETASE GENE FAMILY

Allison Demas, Pamela Magistrado, Sarah Volkman, Ulf Ribacke, Dyann F. Wirth

Harvard School of Public Health, Boston, MA, United States

The remarkable plasticity of the *Plasmodium falciparum* genome allows for adaptation in response to selective pressures and challenges efforts to combat this important human pathogen. Evidence of the adaptive nature of this genome includes the expansion and recent positive selection of the acyl Co-A synthetase (ACS) gene family, which includes four orthologs predicted to activate exogenous fatty acids and play important roles in fatty acid scavenging as well as nine paralogs with unknown function. The evolutionary and functional significance for the expansion of the PfACS9 ortholog to nine paralogs, including PfACS5, is unknown, and we sought to functionally characterize these molecules to understand their biological role in the parasite. We therefore generated parasites with conditional knockdown of PfACS5 and PfACS9, which significantly reduced protein abundance, but led to no difference in intra-erythrocytic parasite growth, under either normal or restricted fatty acid growth conditions. To explore possible neofunctionalization, HA-tagged lines of PfACS5 and PfACS9 were characterized for timing of expression, subcellular localization, and interacting partners. We observed differential localization for these proteins using immunofluorescence assays. Unlike ACS9, ACS5 was clearly exported to the red blood cell cytosol and membrane periphery. Western blots of parasite lysate from subcellular fractions support this differential localization, and show peak expression at 30-36 hours post-

invasion (hpi) for PfACS5 and 38-44 hpi for PfACS9. Exploration of the PfACS interactome through pull down assays further supports distinct functions for these enzymes. We hypothesize that the expansion and recent positive selection of the PfACS gene family are the consequence of metabolic pressures driving parasite evolution, and characterization of this family may identify metabolic chokepoints and potential targets for novel antimalarials.

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EFFECTS OF RBC STORAGE CONDITIONS ON *PLASMODIUM FALCIPARUM* INVASION OF RBCS *IN VITRO*

Morgan M. Goheen, Martha A. Clark, Carla Cerami

University of North Carolina Chapel Hill, Chapel Hill, NC, United States

The *in vitro* culture of *Plasmodium falciparum* in red blood cells (RBCs) is essential to studying the molecular and cell biology of the parasite, however, culture methodologies differ between laboratories. One type of variability arises from RBC source and storage conditions. Recent protocols for standard parasite growth suggest collection and storage of RBCs in acid citrate dextrose (ACD), citrate phosphate dextrose (CPD), or citrate-phosphate-dextrose-adenine (CPDA). Most laboratories routinely culture *P. falciparum* in RBCs for up to 4 weeks after RBC collection, although it is known that freshly donated RBC sustain a higher *P. falciparum* growth rate. It is unknown what step of the *P. falciparum* intraerythrocytic life cycle is impacted by RBC storage. Studies on RBC storage for human clinical use in blood transfusions have revealed a relationship between RBC storage and transfusion complications. Current standards for blood banking involve using CPD for RBC collection, removing plasma and leukocytes, then storing RBCs in saline-adenine-glucose-mannitol (SAGM) for up to 42 days at 4°C. Many RBC storage lesions have been documented in these acidic medias, such as decreased deformability, decreased ATP, decreased 2,3-diphosphoglycerate (2,3-DPG), decreased intracellular potassium, increased intracellular NaCl, oxidative damage, lipid peroxidation, changes in membrane phospholipids, and vesiculation of membranes. Using flow cytometry based assays, we have separately examined the effect of RBC storage conditions and time in storage on overall parasite growth, merozoite invasion of RBCs, and merozoite production. We present results on the effects of storing RBCs after two, four, and six weeks in acid citrate dextrose (ACD) and citrate-phosphate-dextrose-adenine (CPDA), as well as in two other solutions known to maintain RBC integrity, RBC buffer (10 mM HEPES, 12 mM NaCl, 115 mM KCl, 5% BSA) and Alsever's solution.

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DE NOVO ASSEMBLY OF A FIELD ISOLATE GENOME REVEALS A NOVEL *PLASMODIUM VIVAX* ERYTHROCYTE-BINDING PROTEIN GENE

Jim Hester[1], Ernest Chan[1], Didier Menard[2], Odile Mercereau-Puijalon[3], John Barnwell[4], Peter Zimmerman[5], **David Serre**[1]

[1]Cleveland Clinic, Cleveland, OH, United States, [2]Pasteur Institute in Cambodia, Phnom Penh, Cambodia, [3]Pasteur Institute, Paris, France, [4]Centers for Disease Control and Prevention, Atlanta, GA, United States, [5]Case Western Reserve University, Cleveland, OH, United States

Recent sequencing of *Plasmodium vivax* field isolates and monkey-adapted strains enabled characterization of SNPs throughout the genome. These analyses relied on mapping short reads onto the *P. vivax* reference genome generated from a monkey-adapted strain. Any locus deleted in this genome would be lacking in the reference sequence and missed in previous analyses. Here, we report de novo assembly of a *P. vivax* field isolate genome. Out of 2,857 assembled contigs, we identify 362 contigs each containing more than 5 kb of contiguous DNA sequences absent from the reference genome sequence. These novel *P. vivax* DNA sequences account for 3.8 million nucleotides and contain 792 predicted genes. Most of these contigs contain members of multigene families and likely originate from telomeric regions. Interestingly, we identify two contigs containing predicted protein coding genes similar to *Plasmodium* red blood cell invasion proteins. One gene encodes the reticulocyte-binding protein gene orthologous to P. cynomolgi RBP2e and P. *knowlesi* NBPXb. The second gene harbors all the hallmarks of a *Plasmodium* erythrocyte-binding protein but clusters separately from all known *Plasmodium* Duffy-binding protein genes. Additional analyses show that this gene is present in most *P. vivax* genomes and transcribed in blood-stage parasites. Our study complements previous genomic analyses and takes full advantage of sequence data to provide a comprehensive characterization of genetic variations in this important malaria parasite. Further analyses of the protein coding genes discovered have the potential to identify genes influencing key aspects of *P. vivax* biology, including novel mechanisms of human erythrocyte invasion.

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THE AUTOPHAGY PROTEIN PFATG7 IS THE ACTIVATING ENZYME OF THE *PLASMODIUM FALCIPARUM* PFATG8 LIPIDATION PATHWAY AND IS ESSENTIAL FOR NORMAL PARASITE GROWTH

Dawn M. Walker, Maribeth Spangler, Najmus Mahfooz, Katherine A. Kemme, Viral C. Patel, Mark E. Drew

The Ohio State University, Columbus, OH, United States

The *Plasmodium falciparum* genome encodes a limited number of putative autophagy genes, specifically the four genes involved in Atg8 lipidation, an essential step in formation of autophagosomes. In other eukaryotic systems, Atg8 lipidation requires the E1-type ligase Atg7, an E2-type ligase Atg3, and a cysteine protease Atg4. We have confirmed that these four putative *P. falciparum* ATG (PfATG) genes are transcribed during the parasite's erythrocytic stages. We hypothesize that these putative autophagy genes are the essential players of a functional Atg8 lipidation pathway in *P. falciparum.* Recent efforst have focused on dissecting the biochemistry of this pathway. We have genetically engineered parasites to allow for regulatable expression of the activating enzyme PfAtg7. Upon PfAtg7 attenuation, parasites exhibit slow growth in culture, indicating the essentiality of this enzyme for normal parasite growth. We have also modified the PfATG7 locus to introduce a C-terminal hemagglutinin (HA) tag. This has allowed us to immunoprecipitate native PfAtg7 enzyme to confirm its biochemical activity. In an *in vitro* conjugation assay combining native PfAtg7, ATP, and recombinant PfAtg8 followed by non-reducing SDS-PAGE conditions, we detect a PfAtg7-PfAtg8 thioester conjugate at approxiamately 150kDa using anti-PfAtg8. Upon reduction, the 150kDa conjugate is reduced to PfAtg7 and PfAtg8. This ability to form a thioester linkage with PfAtg8 provides evidence that PfAtg7 is in fact the activating enzyme of this pathway. As to translational implications of this research, specific inhibitors have been developed for E1-type ligases, such as the mammalian NEDD activating enzyme, and are currently in clinical trials as anticancer therapeutics. A similar strategy could be employed in the development of specific and selective PfAtg7 inhibitors. If successful, these inhibitors would represent a new class of antimalarials.

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DEVELOPMENT OF A NONRADIOACTIVE HETERODUPLEX TRACKING ASSAY TO MEASURE IN-HOST GENETIC DIVERSITY IN CLINICAL *PLASMODIUM VIVAX* INFECTIONS IN CAMBODIA

Matthew Givens[1], Jessica T. Lin[1], Chanthap Lon[2], Charlotte Lanteri[2], Panita Gosi[2], Oksana Kharabora[1], David Saunders[2], Jonathan J. Juliano[1]

[1]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [2]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

Compared to *Plasmodium falciparum*, relatively less is known about the genetic complexity of *P. vivax* infections. Developing assays which can be used in malaria endemic countries to measure in-host diversity can further research into this neglected species. The Heteroduplex Tracking

Assay (HTA) sensitively detects different parasite variants simultaneously existing within an infected person, but relies on the generation of a radioactive probe, limiting its broad application. We developed a novel non-radioactive, fluorescently-labeled capillary electrophoresis-based HTA to measure multiplicity of infection based upon a region between the interspecies conserved blocks 5&6 of merozoite surface protein 1 (PvMSP-1). This new method relies on visualization of peaks that are detected as a heteroduplex formed by a fluorescently-labeled probe and patient-derived PCR amplicon migrate through a nondenaturing polymer, similar to the readout in microsatellite analysis. The new method was applied to *P. vivax* isolates from 25 patients from Anlong Veng, Cambodia. We found that the number of variants within individual persons ranged from 1 to 5 with a mean of 2.4 variants per sample. Virtual heterozygosity was high at 0.892, suggesting good allelic discrimination at the PvMSP-1 locus. In persons with recurrent *vivax* infections, we found reappearance of identical genetic variants in multiple recurrences, suggesting relapse rather than re-infection. We also found novel variants in these recurrent infections, suggesting that there remain minor variants in the initial *parasitemia* that are undetected, or that there are variants that do not emerge in the initial *parasitemia* but emerge in a relapse. These results are consistent with prior HTA studies from this region and reveal significant in-host malaria diversity in Southeast Asia. This new method will allow HTAs to used in malaria-endemic countries for clinical and research purposes.

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CHARACTERIZATION OF A TYPE 2C PROTEIN PHOSPHATASE IN *PLASMODIUM FALCIPARUM*

Bethany J. Jenkins, Thomas M. Daly, Akhil B. Vaidya, Lawrence W. Bergman

Drexel University College of Medicine, Philadelphia, PA, United States

Signaling pathway components, including kinases and phosphatases, have been a growing area of interest in the pursuit of novel antimalarials. Many have been identified as being more closely related to orthologues found in plants or other lower eukaryotes, making them attractive drug targets. In recent years, divergent kinases and phosphatases have been shown to play essential roles in both the human and mosquito stages of the parasite lifecycle. Type 2C protein phosphatases (PP2Cs) are serine/threonine phosphatases characterized by their magnesium dependence. While ten putative PP2Cs have been found in the *Plasmodium falciparum* genome, only one has been characterized, and is involved in the regulation of transcription and translation. We are investigating a PP2C, PfPP2C-1 (Pf11_0362), which diverges from a group of *Apicomplexa*n PP2Cs and shares closer homology with those of the plant *Arabidopsis thaliana*. Since *Arabidopsis* PP2Cs are critical for growth and stress responses, we are interested in the role of this late stage PP2C in *Plasmodium* schizogeny. We have successfully over-expressed PP2C-1 in *P. falciparum* parasite cultures using the mycobacteriophage recombination system, and found that it localizes to the cytoplasm. While we hypothesized that overexpression of PP2C-1 may lead to deregulation of its signaling pathway, these parasites showed no growth defect or phenotypic differences from wild type parasites. Functional significance of this protein is being assessed through gene knockout and knockdown strategies. We are using co-immunoprecipitation approaches to identify binding partners and potential signaling components of the protein. These studies may reveal hitherto unknown signaling pathways in *Plasmodium* schizogeny, and further define the critical role of PP2Cs in these parasites.

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GENOMIC STABILITY OF PFHRP2, PFHRP3 AND ITS FLANKING GENES OF *PLASMODIUM FALCIPARUM* WILD ISOLATES FROM THE PERUVIAN AMAZON REGION ADAPTED TO *IN VITRO* CULTURES DURING A YEAR

Paola Larrauri, Maria del Carmen Orozco, Jorge Bendezú, Dionicia Gamboa, Joseph Vinetz

Cayetano Heredia University, Lima, Peru

Plasmodium falciparum parasites lacking pfhrp2 and/or pfhrp3 were restricted to laboratory strains which lose those genes during long period *in vitro* cultures. However, wild isolates lacking those genes were also found in the Peruvian Amazon Region as reported in 2010. It is hypothesized that these parasites could be losing both genes spontaneously by mitotic recombination in the asexual stage, similar to laboratory strains during long term cultures. The aim of this study is to assess the presence of *pfhrp2, pfhrp3* and its flanking genes as indicative of the subtelomeric regions stability from chromosomes 8 and 13. Six *P. falciparum* wild isolates were adapted and maintained on *in vitro* cultures during a one year period to simulate mitotic replications of the erythrocytic stages under no selective pressure forces. Previously, all samples (blood spots in filter paper) were characterized by PCR in order to confirm the initial *pfhrp2/pfhrp3* gene profiles: 2 (-/-), 1 (+/+), 2 (+/-) and 1 (-/+). Then, these isolates were maintained in cultures for 12 months and one aliquot per month were used to monitor the presence of these genes (pfhrp2, pfhrp3 and their flanking genes) and for the molecular genotyping using 3 genetic markers *(pfmsp1, pfmsp2* and *pfglurp)* and 14 microsatellites. All cultures maintained the original pfhrp2/pfhrp3 and their flanking genes profiles along the period of study (182.5 generations, 1 generation = 48 hrs. of intraerythocytic cycle). Only 1 isolate presented a switch between the *pfhrp3* profile from its filter paper sample (positive) and all its cultures (negative). The molecular genotyping showed the clonal nature of all the culture samples along the year and allows their monitoring along this period of time as quality control tool. In conclusion it was observed a genomic stability of *pfhrp2/pfhrp3* and their flanking genes in these isolates maintained during one-year *in vitro* culture. The switch in one isolate could be explained by the presence of more than one clone at the beginning of the study that was lost during the culture. Additionally, the classic genetic markers (*msp1, msp2* and *glurp*) were cost-effective and enough to determine the genotype of the isolates and as a quality control tool; but microsatellites brought wider information about those genotypes.

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PREVALENCE AND DISTRIBUTION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY AND MUTANT VARIANTS IN MALARIA PATIENTS FROM CAMBODIA

Panita Gosi[1], Charlotte A. Lanteri[1], Soklyda Chann[1], Darapiseth Sea[2], Sittidech Surasri[1], Saowaluk Wongarunkochakorn[1], Kingkan Pidtana[1], Nillawan Buathong[1], Sabaithip Sriwichai[1], Satharath Prom[3], Char Meng Chuor[2], Youry Se[1], Chanthap Lon[1], David L. Saunders[1]

[1]U.S. Army Medical Component-Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [2]National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia, [3]Royal Cambodian Armed Forces, Phnom Penh, Cambodia

Primaquine is a key component of current malaria control efforts in Southeast Asia. However, the safety of primaquine in patients with glucose-6-phosphate dehydrogenase (G6PD) enzyme deficiency remains a substantial safety concern given current diagnostic limitations in most malaria endemic areas. To better understand potential safety concerns and estimate risks for hemolysis that could result from widespread primaquine use, we evaluated the prevalence of G6PD deficiency, and attempted to characterize common G6PD variants in malaria endemic areas with known multidrug resistance along the Thai-Cambodian border.

We measured the prevalence of G6PD deficiency in a total of 1,188 patients in northern, western, central, and southern Cambodia from 2009 to 2012. Results from a qualitative G6PD fluorescence spot test were compared with a high resolution melting (HRM) real-time PCR method to detect G6PD variants. We developed the HRM assay to probe for single nucleotide polymorphisms (SNPs) associated with five of the most common G6PD variants previously reported in Southeast Asia: Viangchan, Mahidol, Canton, Jiangxi, and Chinese-5. Prevalence of qualitative G6PD deficiency among malaria patients was approximately 12%. HRM analysis revealed that the Viangchan variant, typically associated with moderate to severe (WHO Class II) deficiency, was most prevalent. The relatively high proportion of the population at risk with a mutation associated with moderate to severe G6PD deficiency cautions against widespread, unmonitored primaquine administration in Cambodia. In the absence of G6PD screening, and/or careful monitoring for potential hemolysis in unscreened patients, the risk of serious adverse events is high. Correlation with limited quantitative G6PD-deficiency data is currently underway, and will be presented to help estimate risk in this population, and better inform national malaria drug treatment policy in Cambodia.

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DELIVERY STRATEGIES FOR MASS CAMPAIGNS TO ACHIEVE UNIVERSAL COVERAGE WITH INSECTICIDE TREATED NETS: WHICH WORKS BEST? A MULTI-COUNTRY COMPARISON

Celine Zegers de Beyl[1], Hannah Koenker[2], Emmanuel Obi[3], Emmanuel Adegbe[3], Richmond A. Selby[4], Albert Kilian[5]

[1]Malaria Consortium, London, United Kingdom, [2]Center for Communication Programs, Johns Hopkins University, Baltimore, MD, United States, [3]Malaria Consortium, Abuja, Nigeria, [4]Malaria Consortium, Kampala, Uganda, [5]Malaria Consortium and Tropical Health, Montagut, Spain

The use of insecticide treated nets (ITN) is widely recognized as one of the main interventions to prevent malaria. Mass distribution campaigns are the best approach to rapidly scale up ITN coverage. However, the best strategy to distribute ITN to households is still under debate. Data from 14 post campaign household surveys conducted in Nigeria, Ghana, South Sudan, Senegal and Uganda were merged. These campaigns used a variety of strategies such as stand-alone versus integrated distribution, fixed point versus house to house delivery and targeted or limited versus universal coverage ITN allocation. Survey design and data collection methods were similar across surveys, i.e. representative cross sectional household surveys with a two-stage cluster sampling design and a standard questionnaire. Analysis included 13,901 households and accounted for survey design and sampling probabilities. The main outcome indicators were the proportion of households that received at least one ITN from the campaign and the proportion of households reaching universal coverage on the survey day. None of the ITN campaigns increased the household coverage to the expected target of 80% or more households with sufficient ITN (one ITN for every two people or one ITN per sleeping place). There was no difference in campaign effectiveness comparing various strategies for distribution or delivery, providing that enough ITN are available. There were substantial discrepancies between the quantity of ITN distributed to households and the quantity needed in respect to people or sleeping places, independently of the indicator considered. The effectiveness of ITN campaigns does not depend on the strategy but rather on quality of implementation and ITN availability. Coverage achieved confirms that it is essential to complement mass campaigns with continuous distribution systems to achieve universal coverage targets.

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DOES A TORN LONG-LASTING INSECTICIDAL NET FAIL TO PROTECT CHILDREN FROM MALARIA PARASITEMIA? DATA FROM TWO CROSS-SECTIONAL SURVEYS IN WESTERN UGANDA

Albert H. D. Kilian[1], James Ssekitoleeko[2], Anthony Nuwa[2], Geoffrey Namara[2]

[1]Malaria Consortium and Tropical Health LLP, Montagut, Spain, [2]Malaria Consortium, Kampala, Uganda

Durability of long-lasting insecticidal nets (LLIN) is increasingly coming into focus since longer net survival is associated with significant public health savings. However, there is very little data on the extent to which damages to an LLIN limit its protective effect. In the context of malaria control efforts in Western Uganda, physical condition of nets was measured in a random sample of nets from two representative, cross-sectional surveys in July 2011 and October 2012, 18-30 months after mass distribution of LLIN in the area. From a total sample of 1,598 and 3,938 households 592 and 1,313 nets were assessed for physical integrity respectively using a proportionate hole index as recommended by WHO. Of these nets 818 (43%) had been used the previous night by children under five for whom data on malaria *parasitemia* were also obtained. Physical condition of LLIN was considered to be "good" when the total hole surface area on the net did not exceed 100 cm^2 and as "too torn" when more than 1000 cm^2. The proportion of the 818 nets in "good" condition decreased from 80% for nets less than 6 months old to 63% for nets 1-2 years old (p=0.02) and then stabilized around 50% suggesting that nets were discarded when too torn. Parasite rates in children 0-59 months of age decreased over time from 23% at the first survey to 15% (p=0.07) but did not vary significantly with physical condition (p=0.4) being 13.2% (95%CI 9.2-18.4) for "good" nets, 16.7% (11.7-32.4) for "damaged" nets and 18.3% (9.5-32.4) for nets "too torn". In a logistic regression model of *parasitemia* child age showed to be a significant determinant with an Odds-Ratio (OR) of 1.3 per each additional year (p=0.01) as well as district (p<0.005), current fever (OR 3.5, p<0.005) and second vs.first survey (OR 0.55, p=0.04). However, no increased risk of *parasitemia* was found for "too torn" nets (OR 1.0, p=0.9). These data suggest that, in the setting of Western Uganda, even seriously torn LLIN still provide sufficient protection for children and nets are discarded before they lose their protective effect.

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RISK FACTORS ASSOCIATED WITH MALARIA INCIDENCE AMONG YOUNG CHILDREN AND FEMALE *ANOPHELES* MOSQUITO COUNTS IN KOROGWE, TANZANIA

Jenny Liu[1], Roly Gosling[1], Brittany Zelman[1], Samwel Gesase[2], Ramadhan Hashim[2], Silas Otieno[3], Caroline Maxwell[4], Daniel Chandramohan[4], Teun Bousema[4]

[1]University of California San Francisco, San Francisco, CA, United States, [2]National Institute for Medical Research, Tanga, United Republic of Tanzania, [3]National Institute for Medical Research, Kilimanjaro, United Republic of Tanzania, [4]London School of Hygiene & Tropical Medicine, London, United Kingdom

Several studies conducted in Northeast Tanzania have documented a declining trend in malaria transmission beginning well before malaria interventions were scaled up. One explanation for the decline in malaria may be the changes in socioeconomic conditions associated with economic development, and in particular improvements in house construction materials. This analysis has two main objectives: (1) identify risk factors associated with malaria incidence among young children and (2) identify household and environmental factors associated with mosquito density in and around the home. A particular focus is paid to the housing construction materials as determinants in both analyses. For 435 children enrolled in larger trial of intermittent preventive treatment for malaria in infants in Tanga, North-eastern Tanzania, detailed information on their dwelling characteristics were collected. An index scale of housing structure

quality constructed via principal components analysis was converted to decile units for regression analysis. Ordered logistic regressions were used to predict risk factors for child malaria episodes (none, 1-2, or 3+ episodes) and negative binomial regressions were used to predict risk factors for average female anopheles mosquito counts collected in traps in and around the dwelling. Results suggest that, compared to children who reside in houses with better construction materials, residing in the worst type of house significantly increases the risk of malaria two- to three-fold, even when wealth and rural residence is controlled for. Having ceilings is associated with a significant reduction in female anopheles mosquito counts by nearly half, while having cattle around the house increases mosquito counts. In conclusion, these results corroborate findings from other studies of household and environmental risk factors that show associations between malaria risk to poor housing materials. Interventions to reduce the receptivity of an area or exposure via housing type could help to further reduce malaria transmission.

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SELECTION AND CHARACTERIZATION OF A NEW, NON-MELANISING, LINE OF *ANOPHELES GAMBIAE* REFRACTORY TO *PLASMODIUM FALCIPARUM*

Lenka Richterova, Lisa Ranford-Cartwright

Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom

Anopheles gambiae is a principal vector of *Plasmodium falciparum* malaria in Africa. Some individual mosquitoes within a population are naturally refractory to infection. The only existing refractory line of *An. gambiae* (G3) melanises *P. falciparum* parasites, a refractory behaviour uncommon under natural transmission. Understanding common mechanisms of natural refractoriness could be used for development of transmission blocking vaccines or GMO vector strategies. We have selected a new, non-melanising, refractory line of *An. gambiae* from the outbred Keele line named GU-REF. GU-REF was selected for refractoriness to *P. falciparum* clone 3D7 over 11 generations of selection. At the same time, the GU-CON line was selected at random as a control for inbreeding effects. The refractory line was then tested for genotype specificity, parasite stages affected, timing of blood meal digestion after feeding on infected and uninfected blood, expression of candidate genes previously linked with refractoriness, and fitness parameters (costs of refractoriness). GU-REF mosquitoes exhibit a significantly lower infection prevalence compared to GU-CON and the parent Keele line. The refractory behaviour is not specific to the parasite clone (3D7) used for selection, in that refractoriness is seen to an unrelated parasite, HB3. The refractory mechanism affects the parasite stages before the early oocyst. GU-REF mosquitoes do not appear to exhibit fitness costs associated with refractoriness, as measured by fecundity. Protein digestion of the blood meal is slightly faster in GU-REF after an infectious blood-meal, compared to GU-CON. There is no difference in speed of digestion after a non-infected blood-meal. A new refractory line of *An. gambiae* refractory to infection with *P. falciparum* has been selected. The exact mechanism of refractoriness has not yet been characterised, but could involve the speed of blood-meal digestion or non-melanotic immune responses. The GU-REF line does not appear to have fitness costs associated with refractoriness.

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IMPACT OF INSECTICIDE TREATED WALL LINER ON SCHOOL ATTENDANCE IN RURAL WESTERN KENYA

Elizabeth Glaser[1], Adeyemi Okunogbe[1], Jane A. Odhiambo[1], John E. Gimnig[2], Mary J. Hamel[2], Grant Ritter[1], Peter Otieno[3], George Olang[3], Nabie Bayoh[3], Donald S. Shepard[1]

[1]Brandeis University, Waltham, MA, United States, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Kenya Medical Research Institute (KEMRI), Kisumu, Kenya

Results of a cluster randomized trial in rural western Kenya suggest a new form of malaria prevention, insecticide treated wall liner (ITWL) plus insecticide treated nets (ITNs), provide added benefit over that provided by ITN alone, reducing childhood malaria infection by 38% (95% confidence interval [CI], 23%-50%) overall and by 42% (95% CI=26%-55%) in children aged 5-11 years. Prior studies have shown that malaria adversely affects children's academic performance and school attendance. This supplemental study sought to determine whether children from the ITWL plus ITN (intervention) villages had reduced absenteeism when compared to children from the ITN alone (control) villages.We performed a retrospective analysis of attendance registers for children in standards (grades) 1 through 8 via comparison of school attendance in term 1 of the 2010 academic year (prior to ITWL) to term 3 (after ITWL). Data were available from 6 schools serving children from 8 of the 12 villages in the original trial. Using a multilevel mixed effects difference-in-differences regression model, we explored the effect of ITWL on attendance of pupils from intervention vs. control villages between terms 1 and 3. In order to adjust for registers with missing or incomplete recording of absences,mostly in the last weeks of each 13-week term, we choose to limit analysis to attendance from weeks 1-10 in each academic term. We adjusted for clustering by the inclusion of a village-level variable in the multilevel analysis; other covariates used in the analysis were categorical variables for standard, gender, village,and term. The resulting dataset had 1,126 observations, each representing the percentage of school days attended for one child for weeks 1-10 in a term. Overall recorded attendance averaged 90.1 percent (95% CI 88.9%-91.3%) over weeks 1-10 of term 1, so that recorded absenteeism averaged 9.9 percent. The interaction term in the regression showed that attendance improved (and absenteeism decreased) by 4.7 percentage points (95% CI 1.2%-8.1%) for children in intervention compared to control villages (p=0.008), representing a halving of recorded absences. The main limitation was our inability to confirm the overall accuracy of entries from existing school attendance registers. Nevertheless, these favorable preliminary results suggest a beneficial impact on school attendance from adding ITWL to ITN.

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PLASMODIUM FALCIPARUM GAMETOCYTES INFECTIVITY FROM POST ASAQ (ARTESUNATE AMODIAQUINE) TREATMENT PATIENTS SUPPLEMENTED WITH AZADIRACHTIN-ENRICHED NEEM EXTRACT

Rakiswendé S. Yerbanga[1], Robert K. Ouédraogo[1], Dari F. Da[1], Franck A. Yao[1], Koudraogo B. Yaméogo[1], Leonardo Lucantoni[2], Giulo Lupidi[2], Orazio Taglialatela-scafati[3], Anna Cohuet[4], Annette Habluetzel[2], Jean Bosco Ouédraogo[1]

[1]Institut de Recherche en Sciences de la Santé, Direction régional de l'ouest, Bobo Dioulasso, Burkina Faso, [2]Scuola di Scienze del Farmaco e dei Prodotti della Salute, Università di Camerino, Camerino, Italy, [3]Dipartimento di Farmacia, Università di Napoli "Federico II", via D. Montesano, Napoli, Italy, [4]Institut de Recherche pour le Développement, Unité MIVEGEC (IRD 224- CNRS 5290-UM1-UM2), Montpellier, France

Infectivity of malaria species to the mosquito vector has been investigated in the recent years by scientific community. Patients treated with artemisinin-based combination therapy, artesunate amodiaquine (ASAQ), against *Plasmodium falciparum* malaria produce fast clinical responses to

asexual stage of the parasite; but few data are available for sexual forms (gametocytes) responsible for the malaria transmission to human host to mosquito. In this study, *Plasmodium falciparum* gametocytes from naturally infected human after ASAQ 3-day treatment course in presence of azadirachtin-enriched neem (*Azadirachta indica*) extract were assessed for its infectivity to *Anopheles coluzzii*. *Anophèles coluzzii* females were membrane fed on gametocytaemic blood collected from patients after 3 day ASAQ treatment course and supplemented with azadirachtin-enriched neem (*Aza*) extract. Gametocytes infectivity was evaluated by assessing oocysts prevalence and intensity on mosquito midgut. Oocyst prevalence of 43% (CI_{95} 23-60) and oocyst intensity of 10.78 (CI_{95} 0.0-21.9) were still found after ASAQ treatment. However, a single dose of Aza added to gametocytaemic blood, completely block gametocytes infectivity at 60 ppm and reduce the oocyst prevalence to 98% at 50 ppm. This work demonstrated that after 3-day ASAQ treatment, patients are still able to maintain vector infection. But, single dose of Aza at 50 to 60 ppm will help in preventing mosquito infection and in blocking the malaria transmission.

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IMPACT OF COMMUNITY CHANGE AGENTS ON LLIN NORMS AND USE IN TANZANIA

Marc Boulay[1], Dana Loll[1], Hannah Koenker[1], Michelle Kaufman[1], Rob Ainslie[2], Benjamin Kamala[2], Susan Mlangwa[2]

[1]Johns Hopkins University Center for Communication Programs, Baltimore, MD, United States, [2]Johns Hopkins University Center for Communication Programs, Dar es Salaam, United Republic of Tanzania

Since 2009, Tanzania has distributed 27 million LLINs. According to the 2011-12 THMIS, 91% of households now own at least one ITN and 68% of the population used an ITN the night before the survey. To achieve consistent universal net coverage, program planners need more information on the factors that influence net use in net owning households. Social norms are increasingly recognized as an important determinant of a range of health behaviors, although their role in malaria prevention is not understood. This study investigated the role of a Community Change Agent (CCA) program in affecting social norms related to use of long-lasting insecticidal nets. Since 2008, more than 1800 community members have been recruited and trained to become CCAs and to promote malaria awareness and discussion through community meetings, educational events and household visits. This study randomly recruited 1040 men and women living in the Lindi, Rukwa, and Mwanza regions of Tanzania to participate in a behavioral survey. Overall, 81% of respondents in net-owning households reported that everyone in the household slept under a net during the night before the survey. This outcome was significantly related to perceived social norms ($p<0.001$). Adjusting for background characteristics and number of household nets, universal use in a household increased from 57% in households where the respondent believed few or no households in the community used bed nets to 86% in households where the respondent believed that all households in the community used nets. In addition, controlling for background variables and the actual level of net use in the community, respondents who had interacted with the CCA were significantly more likely to believe that more households in their community used bed nets ($p=0.01$). The results of this study suggest that exposure to a community change agent indirectly affects net use through CCAs' effects on descriptive social norms.

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MALARIA CHEMOPROPHYLAXIS: WHY DON'T THE EXPERTS AGREE? AN INTERNATIONAL OPINION SURVEY

Andy Matheson[1], David Lalloo[2], Colin Sutherland[3], Ron Behrens[1], Christopher Whitty[3], Peter Chiodini[1]

[1]Hospital for Tropical Disease, London, United Kingdom, [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [3]The London School of Hygiene & Tropical Medicine, London, United Kingdom

The use of malaria chemoprophylaxis is central to pre-journey recommendations made to many of our travellers. We struggle to ensure that travellers understand the importance of strict adherence, and all too often medication is taken irregularly or just left at the bottom of the bag. One possible reason for the poor adherence is the wide variation in advice that is given to the public, with different expert groups recommending different drugs or no drugs for travellers with identical itineraries. We undertook a questionnaire survey of experts from around the world involved in the development of National Malaria Guidelines. There is a notable difference in the chemoprophylaxis recommendations in the National Guidelines produced by different jurisdictions. We aimed to find out what evidence is used by the different experts in the development of their guidelines; to better understand the reasons for the wide variation in chemoprophylaxis recommendations. The respondents were also asked what sort of evidence they would prefer to use if ideal data were available. We were unable to detect a marked difference in the evidence used by the different jurisdictions. This was true even when broken down to specific areas, such as that used for India. There are several possible reasons for the variation in recommendations despite using the same evidence base. It may be that we are interpreting the available data differently; it may be that the available data is too poor quality to be of any use; or it may be that we are just collating the wrong data. It may be that we have good data, but are drawn to alternative conclusions by other factors such as medico-legal risk and drug costs. It is difficult to know which recommendations will be proved correct, and the outcomes from the different policies will be fascinating to observe. However a there will need to be a coordinated effort to pool the traveller data from all countries to make sense of the results. The development of an agreed tool to standardise the weighting given to evidence types would give a clear rational for any chemoprophylaxis recommendations. This would, ultimately, improve chemoprophylaxis compliance amongst travellers.

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IDENTIFYING SUBPOPULATIONS LEAST LIKELY TO USE MOSQUITO NETS AFTER MASS DISTRIBUTION CAMPAIGNS: CASE OF KANO STATE, NIGERIA

Elizabeth Ivanovich[1], Jui Shah[2], Yazoume Ye[2]

[1]United Nations Foundation, Washington, DC, United States, [2]ICF International, Calverton, MD, United States

Insecticide-treated net (ITN) ownership and particularly use remain low in many malaria endemic countries in sub-Sahara Africa (SSA). With the shift from target group to universal coverage approaches there is need to ensure effective use of ITNs among all subgroups of the population. Identifying subgroups least likely to use ITNs could inform targeted messaging to improve overall coverage. This study aimed to identify the subgroups least likely to use ITNs after the mass distribution campaigns which took place in May and July 2009 in Kano State, Nigeria. The study used post-campaign evaluation survey data which was collected from October to November 2009. Individuals (3,056) living in households with at least one ITN and sleeping in the households the night before the survey visit (de facto population) were included in the analysis. Pearson Chi-square and Chi-square Automatic Interaction Detector (CHAID) regression were used to identify predictors of ITN use and the subgroups least likely to use ITNs. Eight covariate variables were included in the initial model. Five of these, including sex, age, wealth quintiles, education of the head of the household, and campaign distribution wave were used in the final

model as predicators of ITN use. Overall ITN use was 53% among all participants, and age and sex were good predictors of use. Males aged 15-25 years were the least likely to use ITNs, with a use rate of only 23%, while rates ranged from 46% to 62% among other subgroups. While further qualitative research may provide additional inside, these findings provide useful information for targeted awareness messaging during the mass distribution campaigns of ITN which are being implemented in several countries in SSA.

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EFFECT OF AGE OF ITN OWNED BY HOUSEHOLDS ON MALARIA PARASITE INFECTION AMONG CHILDREN UNDER FIVE YEARS OF AGE IN ANGOLA

Ana C. Franca-Koh, **Yazoume Ye**

ICF International, Calverton, MD, United States

Insecticide treated nets (ITNs) are effective for malaria control and provide protection to individuals living in households that own them. ITNs are manufactured to have a long lasting protective effect; however, the effect of the age of ITNs in households on malaria *parasitemia* is not well documented. This study examined the association between the age of ITNs in households and malaria *parasitemia* among children under five years of age in Angola. ITNs that were obtained shortly before the survey may not protect children from malaria *parasitemia* because the infection may have happened before the acquisition of the net. Conversely, ITNs that were obtained a longer time ago may be less protective due to wear and tear of the net, or reduction in efficacy of the insecticide. We performed a multivariate logistic regression to assess the association between the age of ITNs in households and malaria *parasitemia* among children under five years of age using the 2011 Angola Malaria Indicator Survey. We adjusted for eight potential confounders: sex of child, age of child, mother's level of education, whether the household had been sprayed or not in the previous 12 months, household size, household wealth, area of residence, and malaria epidemiologic zones. Children from households that had owned ITNs for 2-6 months before the survey were significantly less likely to have malaria *parasitemia* compared to those from households without ITNs (OR = 0.28, 95% CI: 0.10-0.84). ITNs that had been owned for 1 month or less, or for more than 6 months, were not protective. These findings provide useful information, particularly when assessing the impact of ITN interventions on the reduction of malaria burden.

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MYANMAR ARTEMISININ RESISTANCE CONTAINMENT (MARC) SURVEY: MALARIA AWARENESS AND PREVENTION

Ohnmar Hlabaw[1], **Celine Zegers de Beyl**[2], Thae Maung Maung[1], Sylvia Meek[2], Krongthong Thimasarn[3], Aye Yu Soe[4], David Sintasath[5], Thar Tun Kyaw[6]

[1]Department of Medical Research (Lower Myanmar), Yangon, Myanmar, [2]Malaria Consortium, London, United Kingdom, [3]World Health Organization, Yangon, Myanmar, [4]Three Diseases Fund, Yangon, Myanmar, [5]Malaria Consortium Asia, Bangkok, Thailand, [6]Vector Borne Disease Control, Ministry of Health, Nay Pyi Taw, Myanmar

Despite anecdotal evidence of declining malaria transmission in some parts, Myanmar has the malaria burden in the Greater Mekong Sub-region. With the emergence of artemisinin resistance in the region, Myanmar is at the forefront of containing and ultimately eliminating artemisinin resistant parasites. In 2012, a malaria survey of households was conducted in the areas of known and suspected artemisinin resistance (Tier 1 and Tier 2) to serve as a baseline for the Myanmar Artemisinin Resistance Containment (MARC) efforts. The study domain included representative populations living in high to moderate malaria risk areas and utilized a multi-stage sampling approach stratified by Tier. Overall, 1992 household respondents were interviewed using standardized and pre-tested questionnaires in line with similar malaria surveys previously conducted in Cambodia and Thailand. Overall, 66.5% (95%CI 62.2 to 70.6) of household respondents understood "mosquito bites" as a mode for malaria transmission and 17.2% (95%CI 14.2 to 20.6) did not mention any transmission mode. Household coverage with at least one mosquito net was 97.5% (95%CI 95.1 to 98.7) and insecticide treated net (ITN) was 35.1% (95%CI 28.4 to 42.4). Lastly, 76.5% (95%CI 72.9 to 79.8) of all people (n = 9408) used a mosquito net the previous night and 15.9% (95%CI 12.4 to 20.3) slept under an ITN. General awareness of malaria was found to be modest; further efforts should be placed on improving community perceptions and behaviors for malaria prevention. Household coverage of ITN seemed insufficient to have an impact on reducing malaria transmission. Considering the high coverage and use of untreated mosquito nets, the national malaria prevention strategy should explore short to medium-term approaches to convert these untreated nets into ITNs and LLINs. For the longer term, demand-driven strategies should be in place to replace current untreated mosquito nets, building on the existing "net culture" in Myanmar.

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INSECTICIDE TREATED NET USE UNDER A COMPREHENSIVE DISTRIBUTION PROGRAM IN KENYA: SUCCESSES AND UNAVOIDABLE SHORTFALLS

Peter S. Larson[1], Noboru Minakawa[2], Gabriel O. Dida[3], Mark L. Wilson[1]

[1]University of Michigan, Ann Arbor, MI, United States, [2]Nagasaki University Institute of Tropical Medicine, Nagasaki, Japan, [3]School of Public Health, Maseno University, Kisumu, Kenya

Insecticide treated nets (ITNs) have proven instrumental against holoendemic malaria. As distribution of ITNs throughout sub-Saharan Africa (SSA) is being scaled up, however, maintaining high levels of coverage over time will be instrumental to sustain current gains. We evaluated the impact of an ITN mass distribution campaign in early 2011to a rural Kenyan community along Lake Victoria. Surveyors collected data on ITN use both before and one year following this distribution. At both times, household representatives were asked to provide a complete accounting of ITNs within the home, including net locations and the ages and genders of people sleeping under them the previous night. Other data on household material possessions, education levels, occupations, and community group memberships were recorded. Patterns of ITN use before and following distribution were compared using spatial and multi-variable statistical methods. At the time of distribution, ~50% of residents reported sleeping under an ITN the previous night, a use rate that rose to 92% one year following mass distribution. However, ITN use varied by age and gender, following a similar pattern both pre- and post-distribution. After infancy, ITN use sharply declined until the late teen years when it began to rise again, plateauing at ~30 years of age. Prior to distribution, socio-economic factors such as parental education and occupation were associated with ITN use. Following distribution, ITN use was similar across social groups. Household factors such as ITN availability and sleeping arrangement negatively impacted use. Our results indicate that mass distribution of ITNs was effective in rapidly scaling up coverage. Free distribution of ITNs using a direct-to-household method can eliminate socio-economic and spatial heterogeneities in ITN possession and use. Age is an important factor in determining consistent ITN use, but problems of sleeping arrangement and ITN disappearance will present a challenge to effective intervention campaigns.

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COST EFFECTIVENESS OF INDOOR RESIDUAL SPRAYING IN NYANZA PROVINCE KENYA

Donald S. Shepard[1], Elizabeth Glaser[1], Aggrey Kihombo[2], Sareh Khoshi[1]

[1]Brandeis University, Waltham, MA, United States, [2]Mzumbe University, Mzumbe, United Republic of Tanzania

From its peak in 2004 to 2010, global malaria mortality fell by 32% from 1.82 to 1.23 million according to a 2012 Lancet paper by Murray et al. The expansion of indoor residual spraying (IRS) and insecticide treated nets (ITN) are considered important contributing interventions to this decrease. However, few empirical studies exist about the cost-effectiveness of IRS as a supplement to ITN, particularly in normal operational programs. We conducted a retrospective cost-effectiveness study of this use of IRS in 2010, combining data from two adjacent districts with perennial malaria transmission (greater Nyando and Rachuonyo) in Nyanza Province, Kenya. We assessed district-level costs by developing spreadsheet templates enumerating the categories of inputs (personnel, recurrent, capital), and the quantities and unit costs of each input within each category. We assessed quantities and unit costs through local and national key informants, consulting catalogs, and checking consistency (e.g. known productivity of spray operators and equipment per operator). We amortized capital inputs based on previous publications of the US President's Malaria Initiative (PMI), and computed cost per person in greater Nyando based on the Kenya census estimates. We estimated clinical malaria cases averted from trial previously published from Rachuonyo, and converted this to discounted life years gained (DLYG) by linking with other previous studies. IRS cost 229 Kenyan shillings (US $3.16) per person in the population, with shares of 22% for personnel, 69% for recurrent cost, and 10% for amortized capital. The breakdown for recurrent costs was 35% for vehicle rental, 27% for insecticide, 31% for personal protective equipment, and 7% for other. Per 100 person years, the combination of IRS and ITN compared to ITN alone reduced infections from 44 to 18, clinical cases from 27 to 9, and added 2.27 DLYG. These give cost-effectiveness ratios of $12 per malarial infection averted, $18 per clinical case averted, and $139 per discounted life year gained--a ratio substantially below Kenya's per capita GDP of $795 (a WHO threshold). Our cost per person covered was about half the median reported from 12 PMI countries ($6.94). While a more systematic addition of national and international overheads would increase costs somewhat, our analysis nevertheless suggests that IRS is a highly cost-effective addition to ITN in this endemic region.

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USE OF DEEP SEQUENCING FOR ASSOCIATION MAPPING OF GENES POTENTIALLY INVOLVED IN PYRETHROID RESISTANCE IN *AEDES AEGYPTI*

R. Patricia Penilla, Farah Zamira Vera, Corey Rosenberg, Karla Saavedra-Rodriguez, William C. Black, IV

Colorado State University, Fort Collins, CO, United States

Identification of target site based-resistance to insecticides has relied on detection of the single target sites mutations known to affect insecticide resistance in the field. With the advent of next generation sequencing we are now able to sample the whole genome association to detect single-nucleotide polymorphisms (SNPs) associated with insecticide resistance. This study seeks to identify SNPs associated with pyrethroid survival in natural populations of *Aedes aegypti* collected in the Viva Caucel and Vergel populations from Yucatan, Mexico. Four library sequences were built from the DNA of 25 mosquitoes. Two replicate libraries contained DNA from mosquitoes that had survived one hour exposure to a predetermined LC_{50} (25 µg a.i./bottle) and two contained DNA of mosquitoes that died from the same exposure. Sequences were obtained from an Illumina HiSeq2000/2500 Sequencer. Alignments of paired read data were run in the NextGene software, interrogating each library sequence with an insecticide resistance library of reference containing 307 genes with 4,039,599 nucleotides. SNPs with coverages <25 or >1000 were excluded as were SNPs that didn't occur in all four libraries. Log Likelihood Ratio Tests were then used to identify SNPs associated with resistance. Novel as well previously identified genes were found to be associated with resistance. Additional libraries are being sequenced to test for associations with deltamethrin exposure, and permethrin exposure in other *Ae. aegypti* field populations.

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INTEGRATED ENTOMOLOGICAL SURVEILLANCE IN ZAMBIA: IMPLEMENTATION OF A PHASED PROGRAM FOR DISTRICT BASED DELIVERY THROUGH ENVIRONMENTAL HEALTH TECHNICIANS

Chadwick Sikaala[1], William Lubemba[2], Musapa Mulenga[1], Mulakwa Kamuliwo[1], Anna M. Winters[2], Daniel Bridges[2], Benjamin Winters[2], Matthew Burns[2]

[1]Zambian National Malaria Control Program, Lusaka, Zambia, [2]Akros, Lusaka, Zambia

Zambia has witnessed a rapid expansion in delivery of insecticidal based interventions such as Indoor Residual Spraying and Long Lasting Insecticidal Nets. Despite intensification of vector control programming, entomological surveillance is conducted sporadically and is geographically limited in coverage. Until now, there has been no routine, decentralized government entrenched longitudinal surveillance system that monitors localized species prevalence and supports routine processing of specimens to measure entomological impact of vector control interventions. A conceptual framework based on phased delivery of individual components of an integrated entomological surveillance system has been designed with supporting tools for districts with ongoing vector control activities. Individual components of the program support training of new and existing recruits, utilization of a standardized field surveillance protocol, data management, intra- and inter-district program performance, species composition mapping, and vector bionomics output associated with local malaria transmission. Nine Environmental Health Technicians who were trained for this program were selected to self-manage surveillance sessions in their respective sentinel sites with Community Health Worker assistance; based on their assessed training performance and their national representation. All sentinel sites were proficient in adopting a standardized collection protocol over multiple months during the wet-season and in yielding specimen data for building localized spatial and temporal species maps and associated bionomics. Findings highlight that the decentralized model of entomological surveillance is an achievable goal for national programs. Further exploration is required to address options that allow for nationally sustainable routes to multiply sentinel sites to ensure comprehensive spatial and temporal mapping of vector species and related parameters and assist the National Malaria Control Centre with evidenced based intervention selection and targeting.

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IS INSECTICIDE-TREATED MATERIAL (ITM) USEFUL FOR DENGUE CONTROL? PERSPECTIVES FROM RANDOMIZED CONTROL TRIALS WITH TREATED CURTAINS AND SCHOOL UNIFORMS

Pattamaporn Kittayapong[1], Phanthip Olanratmanee[1], Suporn Thongyuan[1], Teerasak Laksananan[2], Pongsri Maskhao[3], Peter Byass[4], Valérie R. Louis[5], Duane J. Gubler[6], Annelies Wilder-Smith[4]

[1]Center of Excellence for Vectors and Vector-Borne Diseases, Faculty of Science, Mahidol University at Salaya, Nakhon Pathom, Thailand, [2]Social Medicine Group, Sawanpracharak Hospital, Ministry of Public Health, Nakhon Sawan, Thailand, [3]Faculty of Humanities and Social Sciences, Rajabhat Rajanagarindra University, Chachoengsao, Thailand, [4]Center for Global Health Research, Department of Public Health and Clinical Medicine, Umea University, Umea, Sweden, [5]Institute of Public Health, Heidelberg University Medical School, Heidelberg, Germany, [6]Emerging Infectious Diseases Program, Duke-NUS, Singapore, Singapore, Singapore

Dengue is currently becoming a global public health problem. So far, vector control is the only method used to reduce dengue incidence. Our study aims at finding an alternative solution for dengue control by using insecticide-treated materials (ITM); i.e., treated curtains and school uniforms. Two randomized controlled trials were conducted separately in: 1) an urban city where 2,037 households were used to test ITM curtains, and 2) ten schools with 1,825 enrolled students used to test ITM school uniforms. Evaluation was carried out by entomological parameters and questionnaire interview of participants. The mosquito age was determined by using mosquito population age prediction method which is a tool to determine gene expression of age-related genes. The movement of *Aedes* vectors was evaluated by using sticky mosquito traps. Our results showed a significant reduction in *Aedes* density for both households (p=0.006) and schools (p=0.033) following an implementation. However, for the school trial, an average number of *Aedes* vectors increased after one month due to reduced efficacy of impregnated uniforms after frequent washing. Interestingly, the trial using impregnated curtains showed a trend of declining mean age of *Aedes aegypti*, i.e., 1.2 vs 8.6 days (p=0.0002) in treatment and control areas respectively; and a trend of increased movement of vector populations out of households after a one-year trial, i.e., 11.5% difference in treatment areas while no change was observed in control areas. Surveys with participants showed promising acceptability to the technology. In conclusion, application of ITM in dengue control was useful in either reducing dengue vectors and/or reducing their mean ages, which could have an impact on dengue transmission. We observed a reduction of dengue cases in treatment areas when compared to control areas. Further investigation is needed to decide whether an innovative control method using ITM is practical and effective for a long-term and large-scale implementation.

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ASSESSMENT OF THE INSECTICIDE RESISTANCE STATUS OF *AEDES AEGYPTI* IN LIMA, PERU

Carmen Flores-Mendoza[1], Jorge Alarcon[2], Abelardo Tejada[2], Liz Espada[1], Gabriela Calle[1], Gissella M. Vasquez[1], Roxanne G. Burrus[1]

[1]U.S. Naval Medical Research Unit - 6, Lima, Peru, [2]Instituto de Medicina Tropical Daniel Alcides Carrion Universidad Nacional Mayor de San Marcos, Lima, Peru

In Peru, *Aedes aegypti* was successfully eradicated in 1958 after a 13-year DDT spraying campaign conducted by the Ministry of Health (MoH). However, this mosquito re-infested Lima in 2000, and dengue outbreaks were reported five years later. Current MoH practices for *Ae. aegypti* control consist of temephos applications for larval control and pyrethroid spraying for adult control during dengue outbreaks. Based on the chemical spraying history, evaluating the insecticide susceptibility of *Ae. aegypti* populations from Lima could help prevent potential chemical control failures and guide decisions for an effective mosquito vector control program. Therefore, the objective of this study was to determine the insecticide resistance status of *Ae. aegypti* from northern Lima. Centers for Disease Control (CDC) bottle bioassays were performed using 3-5 d-old F1adults. Insecticide susceptibility was evaluated following CDC diagnostic dose and time for alphacypermethrin, deltamethrin, cypermethrin, and lambdacyhalotrin (10 µg/30 min); permethrin (15 µg/30 min); fenitrothion and malathion (50 µg/30 min); DDT (75 µg/45 min), and benthiocarb (12.5 µg/30 min). *Ae. aegypti* New Orleans and Rockefeller strains were also evaluated and used as reference for insecticide susceptibility. *Ae. aegypti* F1 population from Lima was 100% susceptible to all five pyrethroids and to malathion but resistant to DDT (10%). This population was apparently less susceptible to fenitrothion (78%) and benthiocarb (3%); however, *Ae. aegypti* susceptible strains were also less susceptible to fenitrothion (<14%) and benthiocarb (<2%). Our results suggest that no insecticide resistance exists to the five pyrethroids examined and to malathion, yet this *Ae. aegypti* population is resistant to DDT. The response to fenitrothion and benthiocarb should be re-examined at different diagnostic doses and times to determine if *Ae. aegypti* from Lima are actually resistant to these chemical classes.

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THE PLASTICITY AND HERITABILITY OF SPATIAL REPELLENCY RESPONSES TO TRANSFLUTHRIN IN *AEDES AEGYPTI*

Joseph Wagman, John Grieco, Nicole Achee

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

The potential for spatial repellents to contribute to novel vector control approaches, especially in transmission settings unaffected by traditional tools such as indoor residual spraying and insecticide treated nets, is widely recognized as a research priority. The process of developing new spatial repellent products and strategies has been hampered, however, by the fact that work in this area involves complex behaviors that remain not well defined and/or poorly understood. *In vitro* bio-assays consistently show that disease vectors exhibit a wide range of behavioral responses to repellent chemicals in controlled experimental settings. In order to gain a better understanding of how behavior modification can impact vector populations, the plasticity and heritability of spatial repellency responses in *Aedes aegypti* following exposure to transfluthrin was investigated using a previously described high-throughput bioassay. In general, recently colonized (F_1 generation), non-mated mosquitoes were introduced into an assay system containing a chemical gradient established by dual-ended exposure chambers: a repellent chamber treated with 1.35 mg/m^3 transfluthrin and an untreated control chamber. Mosquitoes that were repelled (moving away from the treated chamber) were considered responders and labeled SRA+, while mosquitoes that were not repelled (remained inactive) were considered non-responders and labeled SRA-. After each evaluation, specimens were collected alive and segregated based on observed behavioral phenotype. We present results on 1) the reproducibility of the behaviors in individual mosquitoes retested after a 48 hour resting period and 2) the heritability of spatial repellent behavior through six generations in which male responders were selectively bred with female responders, and non-responders with non-responders.

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CARBAMATE AND ORGANOPHOSPHATE RESISTANCE IN *ANOPHELES GAMBIAE* ACROSS SOUTHERN GHANA: PATTERNS AND PREDICTION

John Essandoh[1], Alexander E. Yawson[2], David Weetman[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]BNARI, GAEC, Accra, Ghana

Malaria is hyperendemic in Ghana and is a major cause of death and poverty. With strong DDT resistance entrenched throughout much of West Africa, carbamates and organophosphates (OPs) are the preferred alternatives to pyrethroids for IRS. However, resistance to both insecticide

classes has been documented in *Anopheles gambiae* in West Africa: to maintain insecticide efficacy, it is important to predict how and where resistance is likely to occur and spread. *Anopheles* larvae were sampled from 18 sites spanning five distinct ecological zones in southern Ghana from March to Mid-August 2011. Adult mosquitoes were bioassayed with bendiocarb and fenitrothion. Species and molecular characterization were performed using Scott and SINE PCRs respectively. Taqman qPCR assays were used to genotype the ACE-1 G119S resistance-associated locus and ACE-1 alleles were cloned and sequencing to determine possible copy number variation. A higher level of resistance was observed to bendiocarb than fenitrothion, though phenotypes correlated across populations. M-form and S-form were found in sympatry in 15 sample sites but in varying proportions, with three sites harbouring only M-forms. ACE-1 resistant allele (119S) frequency was much higher in S than M forms and a population from Ashiaman, a rice-growing area in Greater Accra, exhibited the highest 119S frequency reported to date (68%). ACE-1 frequency was found to be the strongest independent predictor of phenotypic resistance to both insecticides. However, duplication of ACE-1 was detected, with some individuals displaying multiple distinct alleles. Further work is now required to determine the distribution and resistance-association of ACE-1 duplications in southern Ghana.

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MULTIPLE INSECTICIDE RESISTANCE IN *ANOPHELES GAMBIAE S.L.* ACCORDING TO COTTON CULTIVATION SCHEMES IN BURKINA FASO, WEST AFRICA

Moussa Namountougo[1], Frédéric Simard[2], Abdoulaye Diabaté[1], Thierry Baldet[3], Thibaud Martin[4], Jean Bosco Ouédraogo[1], Géorges Anicet Ouédraogo[5], Roch Kounborbr Dabiré[1]

[1]Institut de Recherche en Sciences de la Santé, Bobo-Dioulasso, Burkina Faso, [2]IRD/MIVEGEC/BEES, Montpellier, France, [3]IRD/CIRAD, Montpellier, France, [4]CIRAD, Montpellier, France, [5]UPB, Bobo-Dioulasso, Burkina Faso

In absence of effective vaccine, the most realistic strategy to control malaria is based on vector control relied on the use of synthetic insecticides. Unfortunately, due to the emergence of insecticide resistance in natural vector populations, many malaria vector control programmes are challenging field control failures. Here, we present new data from Burkina Faso, where longitudinal and cross-sectional surveys were conducted to monitor the frequency of the L1014F kdr and G119S ace-1R mutations and the role of metabolic-based detoxifying mechanisms in contributing to insecticide resistance in field *Anopheles gambiae* populations. In Burkina Faso since 2008 two innovative cotton growth systems based on transgenic (Bt) and biological cotton were introduced using no or less insecticide for pest control prospects. In such context, a country-wide survey associating bioassays and molecular investigations carried out from 2008 to 2010 through 26 localities in Burkina Faso. Populations of *An. gambiae* tested showed during these three years survey a generalising resistance status to PYs (permethrin and deltamethrin) and decreased mortality to bendiocarb whereas they remained susceptible to OP (chlorpyriphos methyl and fenitrothion) irrespective to area. The frequency of the L1014F kdr mutation was highest in the sudan region ranging from 0.75 to 0.99 and relatively moderated in the sudano-sahelian area. Results showed also over-expression of detoxifying enzymes such as GST, oxygenases, cytochrome P450 in *An. gambiae s.s.* from the old cotton belt together with kdr and ace-1R mutations indicating the existence of multi-resistance in Burkina Faso. The geographical distribution of resistance in *An. gambiae s.l.* populations was found in sites of cotton cultivation that has expanded dramatically in the last ten years. Until the discovery of new insecticides or formulations of existing insecticides, it is crucial to integrate the regional vector resistance status in the implementation of control interventions that will preserve a long term efficacy of these vector control tools.

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THE IMPACTS OF VECTOR CONTROL ON THE EFFECTIVE POPULATION SIZES OF MALARIA MOSQUITOES

Giridhar Athrey[1], Theresa Hodges[1], Kevin Deitz[1], Michael Reddy[2], Hans J. Overgaard[3], Abrahan Matias[4], Frances Ridl[5], Immo Kleinschmidt[6], Agalgisa Caccone[2], Michel A. Slotman[1]

[1]Texas A&M University, College Station, TX, United States, [2]Yale University, New Haven, CT, United States, [3]Norwegian University of Life Science, As, Norway, [4]Medical Care Development International, Malabo, Equatorial Guinea, [5]Medical Research Council, Durban, South Africa, [6]London School of Hygiene & Tropical Medicine, London, United Kingdom

The battle against malaria mosquitoes in sub-Saharan Africa is being fought with two main weapons: indoor residual spraying of insecticides (IRS) and long-lasting insecticidal net (LLIN) campaigns. Although many programs have been successful in reducing malaria infection, demonstrating the impact of these programs on vector populations is typically confounded by numerous variables associated with collection methods. Without accurate ways of measuring the impacts of vector control, it is also difficult to determine the optimal frequency for insecticide spraying to keep transmission rates low. Here, we analyzed more than 2,200 samples of three important malaria vectors - *Anopheles gambiae, An. melas*, and *An. moucheti* - from seven sites in Equatorial Guinea that were collected over the course of anti-vector programs in that country (2004-2010). Taking advantage of recently developed coalescent genetic approaches, we addressed two main questions: a) what is the impact of vector control programs on effective population size? and b) how is the effective population size effected by single insecticide spray round? We demonstrate convincingly for the first time that both IRS- and LLIN-based control resulted in dramatically lowered effective population sizes (between 55%-87%) in all populations, with the exception of a single population of *An. melas*. No such reductions were observed in negative control populations. We also found that mosquito populations are dramatically reduced following IRS rounds (65-92%), but rebounded (2,818% increase) between 3-5 months after spraying, indicating that increased spray frequency is likely to greatly improve the impact of IRS on malaria transmission. Our findings are especially important to malaria control because we were able to conclusively link anti-vector interventions to genetic impacts, a linkage that has been difficult to establish in the past.

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QUANTITATIVE AND QUALITATIVE ANALYSIS OF GENE DUPLICATION IN INSECTICIDE-RESISTANT *ANOPHELES* MOSQUITOES FROM WEST AFRICA

Luc S. Djogbénou[1], John Essondah Essondah[2], Edi Constant Constant[2], Keith Steen Steen[2], Benoit Assogba Assogba[3], Roch Dabiré[4], Guillaume Ketoh[5], Martin J. Donnelly[2], David Weetman[2]

[1]Institut Régional de Santé Publique/Université d'Abomey-Calavi and Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [3]Institut Régional de Santé Publique/Université d'Abomey-Calavi, Ouidah, Benin, [4]IRSS/Centre Muraz, Bobo-Dioulasso, Burkina Faso, [5]Université de Lomé, Lomé, Togo

Gene duplication is thought to provide a major source of material for evolutionary innovation. In addition to well-known point mutations that cause Ace-1 target site insensitivity for organophosphate and carbamate insecticides, Ace-1 gene duplication has been found in several West African populations of the primary malaria vector *Anopheles gambiae s.s.* Initially using a PCR-RFLP protocol we recorded excess heterozygosity at the Ace-1 resistance locus (G119S) in several field populations, consistent with the presence of a duplication phenomenon, but specific data on individual specimens could not be inferred. Here, we develop and apply quantitative real-time PCR (qRT-PCR) with SYBR Green detection, alongside both analogue and digital droplet qPCR Taqman assays to investigate

the range of allele-specific Ace-1 copy number variation occurring in natural *An. gambiae* populations in West Africa. Our results reveal that: (a) Ace-1 duplication is widespread across West Africa; (b) is present in unexpectedly high copy number (at least five-fold); and (c) multi-copy resistant homozygotes are not uncommon, despite strong prior evidence for fitness costs in single-copy homozygotes. The pairing of the G119S Taqman assay with our newly-developed copy number qRT-PCR assay provides an informative paired-diagnostic to assess the consequences of Ace-1 mutation and duplication for insecticide resistance phenotypes and fitness in wild *An. gambiae* populations.

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RISE OF MUTATION CYS 1534 IN THE VOLTAGE GATED SODIUM CHANNEL GENE IN *AEDES AEGYPTI* IN MEXICO

Farah Z. Vera-Maloof, Karla Saavedra-Rodriguez, Saul Lozano-Fuentes, William C. Black, IV

Colorado State University, Fort Collins, CO, United States

Aedes aegypti, is the primary vector to humans of dengue and yellow fever flaviviruses (DENV, YFV), and is a known vector of the chikungunya alphavirus (CV). Because vaccines are not yet available for DENV or CV or are inadequately distributed in developing countries (YFV), management of *Ae. aegypti* remains the primary option to prevent and control outbreaks of these arboviral diseases. Permethrin is one of the most widely used active ingredients in insecticides for suppression of adult *Ae. aegypti*. In 2012, we documented a replacement mutation in codon 1,534 of the voltage-gated sodium channel gene (para) of *Ae. aegypti* that encodes an cysteine rather than a phenylalanine and confers resistance to permethrin. A total of 86 field collections containing 4,014 *Ae. aegypti* were made throughout México from 1999 to 2012. These mosquitoes were analyzed for the frequency of the Cys1,534 mutation using a melting-curve PCR assay. Dramatic increases in frequencies of Cys1,534 were recorded from the late 1990's to 2012 in several states including Nuevo Leon in the north, Veracruz on the central Atlantic coast, and Yucatan, Quintana Roo and Chiapas in the south. From 1999 to 2012, the overall frequency of Cys1016 was 0.28. In 2000 in Veracruz the frequency was very low and by 2012 the frequency rose to 0.93. In 2008 Martinez de la Torre and Coatzacoalcos had frequencies of 0.94-1. In 2012 the frequency increased to 0.97 and had become fixed in Tuxpan. The earliest detection of Cys1,534 was in Chiapas, Guerrero and Veracruz in 2000. In total, we document a dramatic increase in the frequency of the Cys1,534 mutation in Mexico from 1999 to 2012. This may be related to previous extensive use of DDT and continued heavy use of permethrin. A rotational schedule utilizing different classes of adulticides should be implemented to slow or prevent fixation of Cys1534.

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BITING BEHAVIOR AND HIGH RESOLUTION MELTING DETECTION OF INSECTICIDE RESISTANCE IN *ANOPHELES GAMBIAE* IN MALI

Moussa Keita[1], Sidy Doumbia[1], Bilkissou Yagoure[1], Amadou S. Traore[2], Adam M. Jenkins[3], Kimberly Regna[4], Seydou O. Doumbia[1], Sekou F. Traore[1], Donald J. Krogstad[5], **Mamadou B. Coulibaly**[1], Marc A. Muskavitch[3]

[1]University of Science, Technologies and Techniques, Bamako, Mali, [2]Tulane University Health Sciences Center, Bamako, Mali, [3]Boston College, Chestnut Hill, MA, United States, [4]Boston College, Boston, MA, United States, [5]Tulane University Health Sciences Center, New Orleans, LA, United States

Understanding the behavior and detecting insecticide resistance in malaria vectors have important implications for malaria control. In 2012-2013, in four traversal passages (start, middle and end of transmission, and dry season), we have collected mosquitoes in two rural areas, Dangassa and Dioro in Mali, using human landing catches. We have used WHO bioassays to detect phenotypic resistance, and high resolution melting (HRM) technology to detect target-site mutation frequencies in the kdr locus. Preliminary results reveal a nearly even split in the proportion of mosquitoes biting indoors vs. outdoors. The proportions were 47.7% vs. 54.3% (n=1013) in July 2012, 55.7% vs. 44.3% (n=517) in October 2012, 49.7% vs. 50.3% (n=155) in December 2012 and 38.0% vs. 62.0% (n=21) in April 2013 in Dangassa. In Dioro, they were 42.8% vs. 57.2% (n=35) in July 2012, 49.7% vs. 50.3% (n=151) in October 2012, 49.7% vs. 50.3% (n=155) in December 2012 and 57.1% vs. 42.9% (n=7) in April 2013. WHO susceptibility assays detected substantial resistance to DDT at both sites (22% and 14% mortality rates in Dangassa and Dioro, respectively) and susceptibility to bendiocarb and pirimiphos-methyl. The HRM analysis for kdr genotypes conducted on a subsample showed frequencies of 0.2 (RR), 0.3 (RS) and 0.5 (SS) at the start of the rainy season, and 0.2 (RR), 0.0 (RS) and 0.8 (SS) during the middle of the rainy season in Dangassa. In Dioro, the kdr allele frequencies were 0.4 (RR), 0.3 (RS) and 0.4 (SS) at the start of the rainy season and 0.4 (RR), 0.2(RS) and 0.4 (SS) during the middle of the rainy season.

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CHARACTERIZATION OF INSECTICIDES RESISTANCE IN *AEDES AEGYPTI* POPULATION FROM THE CARIBBEAN REGION OF COLOMBIA

Ronald Maestre Serrano[1], Doris Gómez Camargo[1], Suljey Cochero[2], Elkin Monterrosa Vergara[3], Hugo Soto Lacouture[4], Zulibeth Florez Rivadeneira[5], Marcelo Torres[6], Sergio Goenaga Olaya[7], Erick Perdomo[8], Brenda G. Silva[9], Adriana E. Flores[9]

[1]Universidad de Cartagena, Cartagena, Colombia, [2]Secretaria de Salud de Sucre, Sincelejo, Colombia, [3]Secretaria de Salud de Córdoba, Montería, Colombia, [4]Secretaria de Salud del Cesar, Valledupar, Colombia, [5]Secretaria de Salud de la Guajira, Riohacha, Colombia, [6]Secretaria de Salud Distrital, Barranquilla, Colombia, [7]Secretaria de Salud del Atlántico, Barranquilla, Colombia, [8]Secretaria de Salud del Magdalena, Santa Marta, Colombia, [9]Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, Mexico

We determined the susceptibility to insecticides and biochemical and molecular mechanisms involved in insecticide resistance of nine populations of *Aedes aegypti* in the Caribbean Region of Colombia. Bioassays were performed for temephos in larvae according to WHO and bottle bioassays for adults with the insecticides: lambdacyalothrin, cyfluthrin, permethrin, deltamethrin, malathion, fenitrothion and pirimiphos-methyl The resistance ratios were calculated using the susceptible Rockefeller strain as a control. Additionally, the organochloride DDT was evaluated through the impregnated papers technique. Biochemical resistance mechanism were identified associated with high level of α, β-esterases, mixed-function oxidases, insensitive acetylcholinesterase and glutathione S-transferases; we identified the mutation Ile1,016 in the gene of the voltage-dependent sodium channel and its frequency. All populations were susceptible to the organophosphates evaluated (RR=1x-4x) with exception of Puerto Colombia and Soledad (Atlántico) strains which demonstrated high and moderate resistance to temephos (RR=15x) and (RR=5x), respectively and Sincelejo (Sucre) with moderate resistance to pirimiphos-methyl (RR=5x). All populations were resistant to DDT (2-28% mortality). Strains evaluated exhibited values of resistance to lambda-cyalothrin between 4,9-83 fold, for deltamethrin between 0,9-37,8 fold, cyfluthrin with 0,5-33,8 fold and permethrin of 1,8 -17,9 fold. Over-expression of glutathione S-transferases were found in all populations with the exception of Puerto Colombia (Atlántico) and Cartagena (Bolivar); as well as α-esterase in strains: Valledupar (Cesar) and Monteria (Cordoba); and insensitive acetylcholinesterase in Puerto Colombia strain (Atlantico). The mutation Ile1,016 was registered in all populations with variability in its frequency.

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HYDROLOGICAL DISTURBANCE AFFECTS COMPETITION BETWEEN *AEDES* VECTOR MOSQUITOES VIA CHANGES IN LEAF LITTER

Paul T. Leisnham, Cassandra Smith, T. Zach Freed

University of Maryland, College Park, MD, United States

The invasive mosquito *Aedes albopictus* utilizes water-holding containers for its development where it competes for food as larvae with the native *Aedes triseriatus* in the eastern United States. We tested the hypothesis that prior hydrological disturbance would affect competition between *Ae. albopictus* and *Ae. triseriatus* in containers via changes in leaf litter decomposition, associated microbial resources, and leached tannins. Containers provisioned with senesced litter were treated to mimic three broad hydrological regimes experienced by containers in nature: dry, flooded, and a wet-dry cycle, before varying densities of competing first-instar *Ae. albopictus* and *Ae. triseriatus* larvae were added using a response surface design. We found that hydrological regime affected litter resource quality, water quality, and *Aedes* competition. Previously dry leaf litter decayed more slowly, supported lower microbial abundance, and leached higher tannin concentrations than litter that had been flooded or exposed to a wet-dry cycle. Containers with previously dry litter experienced more intense competitive effects of *Ae. albopictus* on *Ae. triseriatus* population performance than containers that had previously been flooded or exposed to a wet-dry cycle. In contrast, prior hydrological regime did not affect the population performance of *Ae. albopictus.* These results suggest that prolonged wetter conditions prior to *Aedes* utilization of container habitats may relax competitive effects of *A. albopictus* on *A. triseriatus,* and help foster coexistence between the two species. Coexistence of these *Aedes* mosquitoes has implications for understanding mosquito invasions generally and specific disease risks in eastern North America.

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DESIGN AND TESTING OF A NOVEL, PROTECTIVE HUMAN-BAITED TENT TRAP FOR THE COLLECTION OF ANTHROPOPHILIC DISEASE VECTORS

Benjamin J. Krajacich[1], Jeremiah R. Slade[2], Robert T. Mulligan[2], Brendan LaBrecque[2], Kevin C. Kobylinski[1], Meg Gray[1], Massamba Sylla[1], Wojtek S. Kuklinski[1], Jonathan A. Seaman[1], Brian D. Foy[1], Timothy A. Burton[1]

[1]Colorado State University, Fort Collins, CO, United States, [2]InfoScitex Corporation, Waltham, MA, United States

Currently, there exists a deficit of safe, active trapping methods for the collection of host-seeking *Anopheles* and other disease-causing arthropod vectors. The gold standard approach for mosquito collection is that of Human Landing Catch (HLC) in which an individual exposes bare skin to possibly infected vectors. Here, we present the development of a new method for mosquito collection, the InfoScitex Tent (IST), which utilizes modern tent materials coupled with a novel trap design. This provides an efficacious, non-labor intensive, and safe method for vector collection. In these initial studies, we found it collected an average of 31.5 *Anopheles gambiae s.l.* per trap per night in rural villages in Southeastern Senegal, and 42.5 *Culex* group V per trap per night in the semi-urban town of Kedougou, Senegal. In direct comparisons to HLC, the tent was not statistically different for collection of *Cx. quinquefasciatus* in crepuscular sampling, but was significantly less efficacious at trapping the highly motile dusk biter *Aedes aegypti*. These studies suggest that the IST tent is a viable and safe alternative to HLC for *Anopheles* and *Culex* sampling in areas of high vector-borne disease infection risk.

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HETEROGENEITY IN MALARIA VECTOR DYNAMICS AND BIONOMICS IN NCHELENGE DISTRICT, ZAMBIA

Smita Das

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

As part of the Southern Africa International Centers for Excellence in Malaria Research (ICEMR) project, mosquito collections were performed from March-May 2013 in Nchelenge District, Luapula Province, Zambia. Located along the environs of Lake Mweru and Kenani Stream, Nchelenge experiences hyperendemic transmission and has the highest malaria infection rate in children under the age of 5 years despite implementation of indoor residual spraying (IRS) and long-lasting insecticide-treated net (LLIN) distribution. Center for Disease Control light traps (CDC LTs), pyrethroid spray catch (PSC), and larval collections were performed at three villages along Lake Mweru and two villages along Kenani Stream. The collections revealed that *Anopheles gambiae* sensu stricto is the dominant vector in the lakeside villages, whereas An. *funestus s.s.* is the primary vector with secondary contribution from *An. gambiae s.s.* in the streamside villages. Both human malaria infection rates and vector populations were higher near the streamside villages than those of the lakeside villages. Surveys of potential oviposition sites found that temporary water bodies near the stream and the stream itself are the major breeding sites for *An. gambiae* and An. *funestus*. Both vector species are highly anthropophilic and are predicted to have high sporozoite infection rates. The differences in human malaria infection rates and mosquito abundances between the lake and stream sites support the hypothesis that heterogeneity exists in the human blood index, entomological inoculation rates, and multiple blood feeding behavior of the two vectors within Nchelenge District. The insecticide resistance status of both malaria vectors will also be explored. The vector data in Nchelenge present unique opportunities to further our understanding of malaria transmission and the implications for malaria control in high-risk areas.

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CEMETERIES ARE EFFECTIVE SITES FOR SURVEILLANCE OF LA CROSSE VIRUS AND VECTOR POPULATIONS IN APPALACHIA

Rebecca T. Trout Fryxell[1], Kimberly Freyman[2], Armando Ulloa[3], Brian Hendricks[1], Dave Paulsen[1], Abelardo Moncayo[2]

[1]University of Tennessee, Knoxville, TN, United States, [2]Tennessee Department of Health, Nashville, TN, United States, [3]Regional Center for Public Health Investigation, Tapachula, Mexico

In North America, the mosquito-borne disease La Crosse encephalitis is the leading cause of arboviral disease among children, and was previously limited to the upper Midwest. Unfortunately, the Appalachian region, with Tennessee in particular, now has the highest incidence risk in the nation: 228.7 cases per 100,000 children 15 years and younger, and almost 75% of all US cases reported in a year are in Appalachia (Haddow and Odai 2009). In 2012, nine pediatric cases of La Crosse encephalitis occurred in eastern Tennessee, including one death. While *Aedes* (*Ochlerotatus*) *triseriatus* has been the historical vector, *Ae. albopictus* and *Ae. (Oc.) japonicus* are two invasive species that may be important accessory vectors. All three vectors oviposit desiccant-tolerant eggs in forest stands and opportunistically oviposit eggs in artificial containers. Use of artificial containers may move La Crosse virus (LACv) from the forest's tree holes and into the urban environment as LACv can be transmitted to mosquito offspring (transovarial transmission). In an attempt to detect LACv in active mosquito populations, our objective was to determine if cemeteries were effective sites for monitoring LACv and the vector population; consequently, we conducted an in-depth vector ecology study centered around the 2012 fatal case. Briefly, 38 cemeteries were selected within 10 radial miles of the fatal case. At each cemetery, four ovitraps baited with water and seed germination paper (egg paper) were placed at the four cardinal directions. Egg papers and water were replaced weekly, from 5

Sept. - 3 Oct. 2012, this yielded a total of 760 egg papers. Recovered egg papers (99.3%) were brought back to the laboratory where eggs hatched and adults emerged. Thus far, we have successfully recovered all 3 vector species representing *Ae. (Oc.) triseriatus* (87.6%), *Ae. albopictus* (12.2%) and *Ae. (Oc.) japonicus* (0.2%), and identified four positive pools of *Ae. (Oc.) triseriatus*. This preliminary data indicates cemeteries are effective sites for surveillance of LACv and vector populations.

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ENVIRONMENTAL INVESTIGATION FOLLOWING A LA CROSSE ENCEPHALITIS CASE FATALITY IN TENNESSEE, 2012

Kimberly Freyman[1], Armando Ulloa[2], Brian Hendricks[3], Rebecca Trout-Fryxell[3], Dave Paulson[3], Abelardo C. Moncayo[1]

[1]Tennessee Department of Health, Nashville, TN, United States, [2]Centro Regional de Investigacion en Salud Publica, Tapachula, Mexico, [3]University of Tennessee, Knoxville, TN, United States

La Crosse encephalitis virus (LACV) is an important cause of pediatric encephalitis in the United States. Historically, human cases have been concentrated in the upper-Midwestern states, but in the mid-1990s , the Appalachian region including east Tennessee became a focal point. In 2012, nine pediatric cases of LACV encephalitis occurred in Tennessee, including one death. To detect LACV in the area, oviposition traps, BG sentinel and CDC light traps were placed at forty-nine sites consisting of cemeteries and houses within ten miles of two pediatric infections including the deceased child from September 5 to October 3, 2012. Ninety-one papers have had adults reared and pooled so far. The pools were tested for LACV by real-time RT PCR. Adult collections from BG and CDC traps at house sites were comprised of 36% *Aedes albopictus,* 29% *Culex erraticus*, 13% *Anopheles punctipennis*, 9% *Cx. pipiens*, 8% *Ochlerotatus triseriatus*, 2% *Ae. vexans*, 2% *An. quadrimaculatus* and 1% other species. Adults emerging from cemetery collected egg papers were *Oc. triseriatus* (87.6%), *Ae. albopictus* (12.2%) and *Oc. japonicus* (0.2%). Papers collected from house sites showed *Oc. triseriatus* (54.8%), *Ae. albopictus* (44.6%) and *Oc. japonicus* (0.6%). During the last two weeks, the percentage of *Oc. triseriatus* emerging from the papers decreased; whereas *Ae. albopictus* increased for house and cemetery sites. Of house sites, 50% showed a composition of two species, 37.5% with three and 12.5% with one. Of cemetery sites, 57.7% had a species composition of two, 27% with one and 11.5% with three species. Some (3.8%) cemetery sites did not hatch. To date, 628 pools of mosquitoes have been tested. All 39 pools from BG and CDC trap collections were negative for LACV. Four pools of *Oc. triseriatus* from egg paper collections were LACV positive. The positive pools came from a cemetery site on October 3, 2012. Along with the successful detection of LACV, these findings suggest a temporal and spatial variation in mosquito activity. *Aedes albopictus* may also be more prevalent near homes and *Oc. triseriatus* near cemeteries.

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FIELD EVALUATION OF A PUSH PULL STRATEGY TO CONTROL MALARIA VECTORS IN NORTHERN BELIZE, CENTRAL AMERICA

Joseph Wagman[1], Grieco John[1], Russell King[2], Ireneo Briceño[2], Christopher Martinez[3], Nicole Achee[1]

[1]Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [2]Ministry of Health, Orange Walk, Belize, [3]Uniformed Services University of the Health Sciences, Orange Walk, Belize

Current vector control tools are quickly becoming inadequate for controlling arthropod-borne diseases such as malaria. The reasons for this are complex but combined highlight the need for development of novel approaches to reduce pathogen transmission. Efforts are being carried out to evaluate the use of spatial repellents and mosquito traps in a combined push-pull strategy to reduce the probability of human-vector contact in and around homes. Here, we report on a 16-night, four-arm Latin square experimental hut study in Belize, Central America that evaluated the ability of this approach to reduce densities of two locally relevant malaria vectors, *Anopheles vestitipennis* and *An. albimanus*, from entering the structures . Utilizing a matched-control (untreated) hut, we measured changes in vector entry patterns at huts receiving either indoor repellent alone (1.4 mg/m^3 transfluthrin), outdoor traps (CDC miniature light traps baited with human foot emanations), or both interventions simultaneously. Outdoor light trap yields were also compared between huts with and without repellent. Results show that while light traps alone did not impact mosquito entry into huts, use of repellent alone significantly reduced mosquito entry by more than 60% (± 4%) for both species. The combined intervention did not result in any further reduction of mosquito entry over repellent alone. In fact, while not significant in terms of absolute numbers of mosquitoes entering the huts, a post-hoc Wilcoxon Signed Rank analysis indicates that the presence of a baited CDC light trap outside of a hut may reduce the repellency effect of transfluthrin. Interestingly, use of an indoor repellent did increase the average numbers of *An. vestitipennis* (an endophagic species) captured in outdoor light traps by 50% (±27%), but no corresponding effect was seen with *An. albimanus* (an exophagic vector). These results indicate that while a combined push-pull intervention has the potential to reduce human-vector interactions, the baseline ecology and behaviors of the target vector(s) will influence efficacy.

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INSECT-SPECIFIC VIRUSES DETECTED IN LABORATORY MOSQUITO COLONIES: IMPLICATIONS FOR EVALUATING VECTOR COMPETENCE EXPERIMENTS

Bethany G. Bolling, Nikos Vasilakis, Hilda Guzman, Vsevolod L. Popov, Saravanan Thangamani, Robert B. Tesh

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

In the past 5 years, there has been a dramatic increase in the detection and description of insect-specific viruses found in field-collected mosquitoes. Evidence suggests that these viruses are widespread in nature and many appear to be maintained by vertical transmission (infected female transmits virus to her progeny). Recent studies also indicate that superinfection exclusion (cells infected with one virus are refractory to infection by a second related virus) may occur between some insect-specific viruses with pathogenic arboviruses, thus altering the vector competence profiles of certain mosquito species. In order to evaluate this phenomenon further, we initiated studies to investigate the presence of insect-specific viruses in our laboratory mosquito colonies. Pools containing 50 male and 50 female mosquitoes collected from each colony were homogenized and virus isolation was attempted in Vero (vertebrate) and C6/36 (invertebrate) cell lines. Cell cultures were examined for cytopathic effect and also screened by electron microscopy for the presence of virus-like particles. Total RNA was extracted from C6/36 cell cultures and submitted for deep sequencing with an Illumina platform. Seven out of 14 colonies were found to contain an insect-specific virus. Phylogenetic analyses and serological tests confirmed the presence of previously described insect-specific flaviviruses as well as several novel viruses. The infection rates detected within the infected mosquito colonies were variable. The potential implications of these findings in regards to vector competence studies will be discussed.

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THE EFFECTS OF TRANSIENT IMMUNE ACTIVATION ON TRANSGENIC *ANOPHELES STEPHENSI* FITNESS

Andrew Pike, George Dimopoulos

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

Mosquitoes of the genus *Anopheles* spread the *Plasmodium spp.* parasites responsible for human malaria. Rising resistance and difficulties of distribution have hampered traditional malaria control efforts, which have focused on chemotherapeutic agents to treat cases as they arise and control of mosquito populations through insecticide use, bednets and habitat removal. These issues, coupled with the lack of an effective

vaccine, call for the development of new malaria control methods. Multiple laboratory groups have created transgenic mosquitoes refractory to malaria infection, but no such lines have yet been released as part of a malaria control program. One problem with genetically modified mosquito lines is that they are generally assumed to be less fit than their wild-type conspecifics, which would stop them from replacing the native population and limit their effectiveness. However, previous studies in *Drosophila* and initial data from mosquitoes indicate that temporary immune induction in transgenic insects may have minimal effects on fitness. Therefore, we set out to investigate how short-term induction of the mosquito immune system affects mosquito fitness. We compared various aspects of mosquito fitness, such as; lifespan, fecundity, development time, mating competitiveness, wing length and blood meal consumption in five separate transgenic lines to the same measures in wild-type mosquitoes and have seen few effects. The transgenic lines tested were chosen to test different aspects of transgenesis that may affect fitness, and include the same gene under different promoters, different genes under the same promoter, different isoforms of the same gene and different insertion points of the same construct. These results suggest that the mere presence of a transgene in mosquitoes does not necessarily lead to a large fitness reduction, and indicate that genetically modified mosquitoes may soon be a viable tool for the control of vector-borne diseases.

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MODERATE PRECIPITATION CONDITIONS FAVORED INCREASE OF MOSQUITO POPULATION VECTORS OF VENEZUELAN EQUINE ENCEPHALITIS IN LA ALTA GUAJIRA COLOMBIA

Cristina Ferro, Jorge Luis De las Salas Ali, Ligia Lugo, **Betsy Bello**

Instituto Nacional de Salud, Bogota, Colombia

In the past 17 years none outbreak of Venezuelan equine encephalitis (VEE) has been registered in La Guajira and the disease is no longer a public health priority. However, it is important to consider the last two epidemics that took place in 1992 and 1995 when the virus showed that it was still a threat, after its disappearance was speculated. The objective of this work was to monitorize the precipitation conditions that favor the increase in the populations of mosquito vectors (*Diptera: Culicidae*), in order to assess the entomological risk of virus transmission. Different precipitation periods were studied from September 2009 to August 2012 in La Guajira. Mosquitoes were collected using CDC traps and identified to species. Daily average abundance of female mosquito vectors species collected per night was calculated, and compared to the accumulated precipitation register of the previous 16 days of the collecting day. Vector species, *Aedes taeniorhynchus* and *Psorophora confinnis*, achieved their maximum abundance, when rainfall was moderated between 30 to 60 mm., either heavy (above 80 mm), very low or absence rainfall affected negatively their populations. Both species proved to need specific and slightly different climatic conditions. In conclusion the entomological risk of transmission of VEE increases in the rainy season, particularly at the end of it.

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TEMPORAL CHANGE IN *ANOPHELES DARLINGI* DIVERSITY IN SOME MALARIA ENDEMIC PERUVIAN LOCALITIES

William Lainhart[1], Kyle Nadler[1], Sara A. Bickersmith[2], Marta Moreno[3], Marlon Saavedra[4], Paulo E. Ribolla[5], Joseph M. Vinetz[3], Jan E. Conn[2]

[1]Department of Biomedical Sciences, School of Public Health, State University of New York-Albany, Albany, NY, United States, [2]Wadsworth Center, New York State Department of Health, Slingerlands, NY, United States, [3]Division of Infectious Diseases, School of Medicine, University of California San Diego, San Diego, CA, United States, [4]Asociacion Beneficia-PRISMA, Lima, Peru, [5]Parasitologia, Universidade Estadual Paulista "Julio de Mesquita Filho", Botucatu, Brazil

WHO reported approximately 500,000 malaria cases in the Americas in 2011, of which 22,878 cases were in Peru. In the city of Iquitos in northern Amazonian Peru, *Anopheles darlingi* is the primary malaria vector. The overall aim of this project is to evaluate vector control measures and seasonality by measuring local allelic diversity (*A*) and effective population size (N_E) in *An. darlingi*. Based on earlier studies that detected mid-range N_E and high gene flow in villages surrounding Iquitos, we hypothesize that neither diversity nor N_E will vary among localities seasonally, although this has not been tested previously. A quantitative measure of vector control effectiveness is a significant reduction in N_E and *A*. *An. darlingi* microsatellite data from fifteen *loci* were analyzed for four localities south and west of Iquitos: San Jose de Lupuna (LUP), Villa El Buen Pastor (VBP), Cahuide (CAH) and Santo Tomas (STO). Genetic diversity, differentiation (F_{ST}), N_E, departures from Hardy-Weinberg equilibrium and linkage disequilibrium were measured for 17-50 specimens per collection. Sequential Bonferroni corrections minimized multiple testing biases. Both structure and F_{ST} analyses detected one population of *An. darlingi*, similar to findings of Mirabello et al. (2008). However, in the current study, N_E estimates were lower. Overall genetic diversity was high and similar for the four localities. These results differ from those of Pinedo-Cancino et al. (2006) who used amplified fragment length polymorphism analyses and reported limited diversity in *An. darlingi* in the same region. Differences between February (dry) and April (rainy) in 2011 were only assessed for LUP and VBP. In this analysis, mean *A* for all *loci* was stable in both localities, but in VBP, observed heterozygosity (H_O) decreased and expected heterozygosity (H_E) increased. Our results suggest local seasonal environmental changes may influence diversity within this population of *An. darlingi*.

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A PHARMACOLOGICAL APPROACH TO VECTOR CONTROL VIA THE *ANOPHELES GAMBIAE* SEX PEPTIDE RECEPTOR

Kimberly Regna[1], Jamie R. Doyle[2], Alan S. Kopin[2], Marc A.T. Muskavitch[1]

[1]Boston College, Chestnut Hill, MA, United States, [2]Tufts Medical Center, Boston, MA, United States

While many vector-targeted control strategies aim to decrease vector survival, there are many mosquito behavioral processes that could serve as targets for strategies that would decrease vectorial capacity. Among such behaviors, mating is a potential target for intervention in many insects, including *Anopheles gambiae*, because there is a dramatic increase in female refractoriness to mating after a single initial mating event. In other insects, including D. melanogaster, sex peptide receptor (SPR) has been shown to play a significant role in regulating mating behavior. SPR is a G protein-coupled receptor that is activated by sex peptide (SP), which is present in the male seminal fluid, and by myoinhibiting peptides (MIPs), which are thought to be ancestral ligands for SPR-related receptors. We are investigating the pharmacology of SPR in *An. gambiae* by screening selected MIPs in cell-based assays to identify receptor agonists. Using an agonist-based approach, it may be possible to induce female refractoriness to mating by delivery of these peptides *in vivo*, and thereby decrease

reproductive ability and fitness of agonist-treated mosquitoes, leading to source reduction. We have found that RNAi-based knockdown of the D. melanogaster SPR ortholog leads to pre-adult developmental arrest and impaired flight ability, suggesting that SPR antagonists may be of interest for control of *An. gambiae* if the mosquito receptor plays similar roles. The goals of this project are to better understand SPR receptor-ligand activity relationships and to investigate the role of SPR in mosquito behavior and survival, in an effort to validate novel drug targets for development of next-generation insecticides.

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FUNCTIONAL CONFIGURATION OF METAGENOME IN THE MOSQUITO GUT ECOSYSTEM

Jiannong Xu[1], Phanidhar Kukutla[1], Hongmei Jiang[2], Lingling An[3], Jinjin Jiang[1], Matthew Steritz[1], Wanqin Yu[1], Celeste Alvarez[1], Thomas Gilbreath[4], Guiyun Yan[4]

[1]New Mexico State University, Las Cruces, NM, United States, [2]Northwestern University, Evanston, IL, United States, [3]University of Arizona, Tucson, AZ, United States, [4]University of California Irvine, Irvine, CA, United States

Host associated microbes are ubiquitous, yet our understanding of the interactive relationships is very limited. The mosquito gut ecosystem accommodates a complex microbial assemblage. The dynamic gut microbiome profoundly affects various mosquito life traits, such as fecundity and immunity. Besides, bacteria may directly interfere with malaria *Plasmodium* development in the gut before invasion occurs. However, little is known about the genetic structure and functional repertoire of the gut microbiome. In this study we generated 15Gbp metagenomic DNA- and RNA-seq data from the guts of adult mosquito *Anopheles gambiae* under conditions with sugar meals or blood meals. Using an assembly-based pipeline, a 37.1 Mbp metagenomic reference was compiled, which included 49,000 contigs. Similarity based taxonomic classification recognized at least 6 phyla, predominant taxa included Proteobacteria (Enterobacteriacea, Psedomonadaceae and Acetobacteracea) and Bacteroidetes (Flavobacteriaceae). The function annotation was implemented via SEED/Subsystems and COG/KEEG, which recognized 23,550 coding sequences. Among them 42% were assigned into ~700 subsystems. Metabolic reconstruction predicted 1658 reactions and 1266 compounds. In addition to the presence of many ABC transporters, there are large numbers of TonB dependent transporters and polysaccharide utilization *loci*, constituting uptake systems for iron, vitamin B 12 and various biopolymers. The presence of large capacity of resistance to antibiotics and toxic compounds may represent a defense strategy for maintaining community stability. The metagenomic reference was further used for mapping RNA-seq reads to decipher context dependent community functions, which was exemplified by metatranscriptomic analysis of the sugar-fed and blood-fed guts. The metagenomic reference provides insights into the taxonomic and functional configuration in the mosquito gut ecosystems.

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DIVERSE SYMPATRIC MALARIA VECTOR SPECIES IN PURSAT PROVINCE, WESTERN CAMBODIA, AN AREA WHERE ARTEMISININ-RESISTANT *PLASMODIUM FALCIPARUM* IS HIGHLY PREVALENT

Becky A. Miller[1], Brandyce St. Laurent[1], Men Sari[2], Chanaki Amaratunga[1], Seila Suon[2], Duong Socheat[2], Robert W. Gwadz[1], Rick M. Fairhurst[1], Jennifer M. Anderson[1]

[1]National Institutes of Health/National Institute of Allergy and Infectious Diseases/Laboratory of Malaria and Vector Research, Rockville, MD, United States, [2]National Center for Parasitology, Entomology and Malaria Control Program, Phnom Penh, Cambodia

Anopheles mosquitoes from a two-year longitudinal entomological collection in Thmar Da commune, Pursat Province, were analyzed to determine which species transmit malaria to humans along Cambodia's border with Thailand. This region has been a hotspot for the evolution of drug-resistant *Plasmodium falciparum* parasites for decades, so understanding the complex transmission dynamics and the vector species responsible for spreading these parasites is critical for effective malaria prevention, control, and eventual elimination. Using human landing catch and CDC light trap methods, we collected 4,264 anophelines comprising 14 different morphologically-identified species (*An. barbirostris, An. dirus, An. hyrcanus, An. hyrcanus group, An. jamesii, An. karwari, An. kochi, An. maculatus, An. minimus, An. nigerrimus, An. philippinensis, An. tessellatus, An. umbrosus*, and *An. vagus*), all of which have been incriminated as (mostly secondary) malaria vectors elsewhere in southeast Asia. Specimens were analyzed for (i) *Plasmodium* infection using a nested PCR, (ii) bloodmeal source (i.e., human or domestic animal), and (iii) the presence of cryptic molecular species defined by rDNA ITS2 *loci*. Preliminary molecular speciation reveals even more species diversity in this area, with multiple cryptic species present. Several different anopheline species, including *An. maculatus*, *An. dirus* A, and *An. tessellatus*, were found to carry *Plasmodium* parasites. The implications of multiple vector species and their biting behaviors for malaria control and transmission in this region will be discussed. The diversity of vector species in Thmar Da and elsewhere in Cambodia is a challenge for vector control efforts and underlies the need for further characterization of vector ecology, behavior, and population genetics in this country's malaria-endemic areas.

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IDENTIFICATION OF *AEDES AEGYPTI* IMMUNE RESPONSES MECHANISMS TO DENGUE VIRUS

Paola A. Caicedo[1], Clara B. Ocampo[1], Carl Lowenberger[2], George Dimopoulos[3], Neal Alexander[1], Idalba M. Serrato[1]

[1]Centro Internacional de Entrenamiento e Investigaciones Médicas-CIDIEM, Cali, Colombia, [2]Simon Fraser University, Burbaby, BC, Canada, [3]Johns Hopkins University, Baltimore, MD, United States

In recent years there has been considerable progress in our knowledge of dengue, particularly in vaccine development, the characterization of the immune responses and molecular properties of the virus. However, there are still many aspects that must be investigated in terms of its transmission by the principal vector, *Aedes aegypti*. We have conducted research to elucidate Dengue virus-vector relationships, specifically the innate immune response of *A. aegypti* to dengue virus infection. For this, we identified and selected two strains of *A. aegypti*, from Cali, Colombia with different susceptibility to dengue infection: Susceptible (Cali-S) and refractory with midgut infection barrier (Cali-MIB). We compared the global gene expression of the midguts of Cali-S and Cali-MIB after ingestion of sugar, a bloodmeal, or a bloodmeal containing Dengue-2 virus using microarrays. Preliminary results from the microarrays indicated the expression of a total of 3761 genes. Of these, a total of 165 immune-related genes have been identified. A differential expression between the two strains exposed to DENv-2 virus included genes in different functional groups; immunity, metabolism, proteolysis, redox, replication, transport, and unknown function. Characterization of these genes is underway to elucidate if refractoriness is related to an upregulation or downregulation of specific or multiple genes in the two strains. This study will provide a global overview of gene expression in susceptible and refractory mosquitoes and will be compared with other studies that have looked at specific molecules and pathways. This study will also validate the use of our field derived strains as an important biological model to study Dengue-vector relationships.

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OCCURRENCE OF NATURAL *ANOPHELES ARABIENSIS* SWARMS IN AN URBAN AREA OF BOBO-DIOULASSO CITY, BURKINA FASO, WEST AFRICA

Kounbobr R. Dabiré[1], Simon P. Sawadogo[1], François de Sales D. Hien[1], Hamidou Maïga[1], Thierry Baldet[2], Frédéric Simard[3], Abdoulaye Diabaté[1], Louis-Clement Gouagna[4], Gabriella Gibson[5], Rosemary S. Lees[6], Jéremie Gilles[6]

[1]Centre Muraz/IRSS, Bobo-Dioulasso, Burkina Faso, [2]Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Montpellier, France, [3]UMR-MIGEVEC, IRD, Montpellier, France, [4]UMR-MIGEVEC, IRD, Ile de la Reunion, France, [5]Natural Resources Institute/University of Greenwich, Central Avenue Chatham Maritime Kent, United Kingdom, [6]Insect Pest Control Laboratory, FAO/IAEA, Vienna, Austria

The swarming behavior of natural populations of *Anopheles arabiensis* was investigated by conducting transect surveys on 10 consecutive days, around dusk, from March to April and from September to October 2012 in Dioulassoba, a district of Bobo-Dioulasso city in Burkina Faso (West Africa). Swarms were observed outside, around identified larval breeding sites on the banks of the Houet River, as well as in the open-air courtyards found at the centre of many homes in the region. Swarms were found to occur in open sunlit spaces, mostly located above physical or visual cues somehow visually distinct from the surrounding area. Overall 67 and 78 swarms were observed, respectively, during the dry season (March-April) and the rainy season (September-October) of 2012, between 1.5 and 4.5 meters above the ground at their centre. 964 mosquitoes were collected and analyzed from dry season swarms, of which most were male, and all were *An. arabiensis*, as were the few resting mosquitoes collected indoors. Larvae collected from breeding sites found on the banks of the Houet River mostly consisted of *An. arabiensis* and only a minority *An. coluzzii* (formerly identified as *An. gambiae* M form). Of 1694 mosquitoes analyzed from 78 swarms in the wet season collections, a few *An. gambiae* males were identified, and the remainder was *An. arabiensis*. The majority of larvae collected during the wet season from the same breeding sites were identified as *An. arabiensis* and only a minority *An. coluzzii* form and even fewer *An. gambiae* (formerly known as *An. gambiae* S form). The same pattern of species composition was seen in resting mosquitoes, though the proportion of *An. arabiensis* was less overwhelming. These data support the conclusion that *An. arabiensis* is the most prevalent species in this area, though the difference in species composition when using different population sampling techniques is noteworthy. Further studies are required for more detailed investigation of male dispersal, feeding behaviour and mating patterns in an urban setting.

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ACCURATE SPECIES IDENTIFICATION IS CRITICAL FOR MALARIA CONTROL: THE UTILITY OF MOLECULAR CHARACTERIZATION OF ANOPHELINE SPECIES ACROSS INDONESIA, A COUNTRY OF DIVERSE VECTORS AND MALARIA TRANSMISSION

Brandyce St. Laurent[1], Puji B. Asih[2], Siti Zubaidah[2], Sumardi S.[3], David Bretz[4], Hellen C. Miller[4], John Mueller[4], William A. Hawley[5], Supratman Sukowati[3], DIn Syafruddin[2], Frank Collins[4], Neil Lobo[4]

[1]Eck Institute for Global Health, University of Notre Dame, South Bend, IN, United States, [2]Eijkman Institute of Molecular Biology, Jakarta, Indonesia, [3]National Institute of Health Research and Development, Jakarta, Indonesia, [4]University of Notre Dame, South Bend, IN, United States, [5]Unicef, Jakarta, Indonesia

Identification of malaria vectors is critically important for the evaluation of malaria transmission dynamics. In areas of high biological diversity, morphological species identification may not fully describe the amount of variation that is relevant to malaria transmission. The burden of malaria in Indonesia, a region of high biological and geographical diversity, is significant and varies across the archipelago, largely due to differences in the types of mosquito species inhabiting each region. In many areas in Indonesia, there are multiple sympatric anopheline species whose specific bionomic traits ultimately determine the dynamics of malaria transmission. Most of these species are isomorphic members of cryptic species complexes. In this study, we used molecular tools to identify anopheline specimens collected from four different sites in Indonesia to molecular species to address site-specific species identification issues as they relate to malaria control. Specimens were collected from four field sites in Indonesia: a low transmission field site in Purworejo, Central Java; a medium transmission field site in Lampung, Sumatra; and high transmission sites in South Halmahera and Papua. 2,840 anopheline samples from different entomological collections, representing 18 different morphological species, were sequenced for ribosomal DNA ITS2 using Sanger sequencing. Molecular species identification revealed 22 different molecular species, a high level of misidentification, and 9 species carrying *Plasmodium falciparum* or *P. vivax* sporozoites. These species include: *Anopheles aconitus, An. balabacensis, An. farauti 4, An. indefinitus, An. kochi, An. maculatus, An. sundaicus A, An. vagus,* and *An. vanus*. Accurate species identification is cost-effective for control programs and site-specific evaluation of species compositions at the molecular level is recommended prior to the implementation of any control or monitoring program. These results will contribute to our understanding of the distribution of vector species, their behavioral patterns, as well as provide new diagnostic tools.

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PRESENCE OF *AEDES (STEGOMYIA) ALBOPICTUS* (SKUSE,1894) (*DIPTERA: CULICIDAE*) IN COLOMBIA

Betsy Bello, Ligia Lugo

Instituto Nacional de Salud, Bogota, Colombia

Aedes (Stegomyia) albopictus (Skuse, 1894), it is the dengue´s vector, yellow fever in Southeast Asia and others arbovirus such as chikungunya fever; this vector is an invasive specie that has the ability to reproduce in natural and artificial environments with a widely geographic distribution, being in different countries in Europe, Africa and America. The first record of *Ae. (Stg.) albopictus* in South America was at Brazil in 1986, followed by Bolivia, Colombia, Paraguay, Argentina, Uruguay and Venezuela. Also, inside of the entomological surveillance that is made in Colombia to exotic species of public health importance, we includes the sentinel surveillance sampling is performed in larvitraps and in some cases ovitraps at strategic points such as airports, land and river ports. Being the first record of *Ae. (Stg.) albopictus* in Leticia – Amazonas (Colombia) in 1998, this place is the border zone with Tabatinga-Brazil and Caballo Cocha, Iceland and Santa Rosa - Perú; after that we found this new vector in six of the thirty-two Colombian departments, starting with the Special District, Industrial, Port, and Ecotourism Biodiversity Buenaventura-Valle del Cauca, 2001. Although there are few records of its role as a vector in the Americas, there is one report of natural infection in *Ae. (Stg.) albopictus* with serotypes Den-1 and Den-2 in Colombia in 2006, specimens from the municipality of Buenaventura, Valle del Cauca. Therefore it is likely that this mosquito in the future become a efficient vector of dengue and other arboviruses in our country continue to be important sentinel surveillance through larvitraps and ovitraps and integrate the control of *Aedes (Stegomyia) aegypti* and *Aedes (Stegomyia) albopictus* in the country, as globalization has enabled the generation of new trade routes and through passive transport in less time get more places both the mosquito vector and the virus and the sick.

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TUBERCULOSIS IN LAMBARÉNÉ, GABON: FIRST EPIDEMIOLOGICAL AND MICROBIOLOGICAL DATA

Sabine Bélard[1], Jonathan Remppis[1], Davy U. Kombila[1], Saskia Janssen[1], Matthias Frank[2], Abraham S. Alabi[1], Bertrand Lell[1], Peter G. Kremsner[2], Martin P. Grobusch[3]

[1]Centre de Recherches Médicales de Lambaréné, Lambaréné, Gabon, [2]Institute of Tropical Medicine, Tübingen, Germany, [3]Center for Tropical Medicine and Travel Medicine, Department of Infectious Diseases, Division of Internal Medicine, Academic Medical Center, Amsterdam, Netherlands

The central African region is highly affected by the pandemics of HIV and tuberculosis (TB), but systematic data on local epidemiology and drug resistance are scarce, if ever available. The objective of this first prospective observational cohort analysis of 200 TB patients in Lambaréné, Gabon, is to describe demographic, clinical and microbiological characteristics and evaluate treatment outcomes. Patients from three different treatment centers in Lambaréné were included and followed up 2 and 6 months after treatment initiation. Sputum samples were sent to Germany for culture and drug sensitivity testing. To date 120 patients have been included; 74 (62%) were male and 20 (17%) were children. 105/120 (88%) were new TB cases, in 9/120 (8%) and 5/120 (4%) retreatment was started due to default and relapse, respectively. Among the adult patients 75/100 (75%) presented with smear positive pulmonary TB, 18/100 (18%) with smear negative pulmonary TB and 7/100 (7%) with extra-pulmonary TB. HIV co-infection was confirmed in 36/120 (30%), in 12/120 (10%) HIV status was unknown. Of the 54 positive sputum culture results obtained so far 47/54 (87%) were identified as *Mycobacterium tuberculosis* and 7/54 (13%) as *M. africanum*. Full drug sensitivity for the first-line antituberculous drugs (RHZES) was ascertained in 44/54 (81%) patients. Resistance to at least rifampicin and isoniazid (multi-drug resistance, MDR) was found in 3/54 (6%), and mono-resistance to isoniazid and streptomycin in 3/54 (6%) each, and combined resistance of isoniazid plus streptomycin in 1/54 (2%). So far treatment outcome could be evaluated for 36 patients; 17/36 (47%) were classified cured, 3/36 (8%) defaulter, 1/36 (3%) treatment failure, 10/36 (28%) lost to follow-up, and 5/36 (14%) deceased. All deceased patients were HIV co-infected. These first interim results indicate that in Gabon TB is a serious public health threat with a high mortality in HIV co-infected patients and a low cure rate. Besides improvement in basic TB control, implementation of mycobacterial culture and drug sensitivity testing beyond research purposes as well as the establishment of a second-line regimen are urgently needed to halt the further spread of MDR TB.

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IMPACT OF RESPIRATORY ILLNESSES DURING PREGNANCY ON NEWBORN'S WEIGHT - A COMMUNITY BASED LONGITUDINAL STUDY AT AN URBAN SLUM IN PAKISTAN

Asad Ali[1], Umber Zaman[1], Samana Zaidi[1], William Petri[2], Zulfiqar Bhutta[1], Anita Zaidi[1], Molly Hughes[2]

[1]Aga Khan University, Karachi, Pakistan, [2]University of Virginia, Charlottesville, VA, United States

Birth weight is a powerful determinant of an infant's long term growth and survival. Although maternal health is widely believed to impact the birth weight of the baby, the exact factors during pregnancy which influence the birth weight are not clearly known. We are conducting a longitudinal observational study at Bilal Colony, a semi urban area of Karachi, Pakistan to assess the effect of maternal morbidities on the weight of the newborn. We are following 400 pregnant women from the first trimester onwards until their delivery. The pregnant women are visited weekly to record any fever or respiratory symptoms during the past seven days, and are referred to the study site clinic for treatment of observed illnesses. Each symptom episode is defined as one or more days of a self-reported symptom (fever, cough, difficulty breathing, runny nose, sore throat, head ache, chills or myalgia) in a pregnant woman who was symptom free for three days before. So far, 288 pregnancies have concluded as live deliveries, 12 as still births and 31 as spontaneous abortions. We analyzed the data of 243 pregnant women whose newborns were weighed within 14 days of birth. The average age of pregnant women in our study was 24.1years and average weight of the pregnant woman was 56.1kg at the time of enrollment. Only 31% of the mothers had primary education or above whereas 38.3% had antenatal visits during their pregnancy. There were 51(21%) newborns with low birth weight (< 2.5 kg), whereas 192(79%) had normal birth weight (>= 2.5kg). In pregnant women who had a low birth weight baby, the average episodes of fever, cough, headache and myalgia were 1.7, 2.3, 4.3, and 4.3 per women respectively. In pregnant women who had a normal birth weight baby, the average episodes of fever, cough, headache and myalgia were 1.7, 2.1, 5.2 and 4.6 per women respectively. The results of this study will help identify the degree to which maternal respiratory illnesses during pregnancy are a risk factor for infant's low birth weight.

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MOLECULAR DETECTION OF HUMAN METAPNEUMOVIRUS ON NASOPHARYNGEAL SWABS COLLECTED FROM OUTPATIENTS WITH ACUTE RESPIRATORY TRACT INFECTIONS FROM MBAGATHI DISTRICT HOSPITAL, KENYA IN THE YEAR 2008

Rosemary M. Nzunza[1], Wurapa Eyako[1], Kariuki Njenga[2], Ongus Juliette[3], James Njiri[1], Berhane Assefa[1], Wallace Bulimo[1]

[1]U.S. Army Medical Research Unit-Kenya, Nairobi, Kenya, [2]GDD/IEIP-Kenya, Nairobi, Kenya, [3]Institute of Tropical Medicine and Hygiene, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

Human metapneumovirus (hMPV) is a leading cause of acute respiratory tract infection in children the elderly and immune compromised persons. In Kenya the extend of hMPV infections in the population remains is unknown. A retrospective study was conducted in the year 2008 in outpatients from ≥2 months of age presenting at the outpatient department of Mbagathi District Hospital for acute respiratory infection. Nasopharyngeal swabs were systematically tested for human metapneumovirus and other respiratory viruses, using real time reverse transcriptase PCR. Epidemiological and clinical characteristics of hMPV-infected children were studied and compared to those of patients with respiratory syncytial virus (RSV) and other viral infections. A total of 498 patients were enrolled in this study. Viral investigations detected a total of 271 viruses. Out of these, 77(15.5%) were hMPV infections, 78(15.7%) Seasonal FluA, 60 (12%) Seasonal Flu B, 13 (2.6%) Panenterovirus, 36(7.2%) Para Influenza viruses and 6(1.2%) RSV infections. Human metapneumovirus infections were higher in males 43(55%) than females 34(45%), and predominantly in children ≤5yrs (97%), only 2(3%) aged between 6-9yrs. The hMPV infection had peak in January-February, and was uncommon after March. Most of the patients infected with hMPV were under 1 year of age and cough (100 %) and difficulty in breathing (75%) were the predominant diagnosis in these patients with clinical symptoms of a lower respiratory tract infection. The severity of the disease was similar to those of RSV patients. These results highlight that hMPV plays an important role in acute respiratory tract infections especially in children. Rapid detection to identify specific viral pathogens causing respiratory tract infections in the wider Kenyan population could aid in patient management.

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MOLECULAR CHARACTERIZATION OF HUMAN ENTEROVIRUS 68 ISOLATED IN KENYA DURING 2008 TO 2010

Silvanos M. Opanda

United States Army Medical Research Unit-Kenya (USAMRU-K), Nairobi, Kenya

Human enterovirus 68 (HEV-68) is a rarely detected viral pathogen associated with acute respiratory illness. It is unique among enteroviruses because it shares common biological properties with rhinoviruses. The

virus was first isolated in California, USA in 1962 and has ever since been identified almost exclusively in respiratory samples. HEV-68 infection is associated with several disease manifestations ranging from mild respiratory illnesses to severe acute lower respiratory tract infections including pneumonia, wheezing and bronchitis. During the period 2008 to 2010 an upsurge in the number of clusters of acute respiratory illness associated with HEV-68 was reported in many parts of the world including Asia, Europe and the United States. Human respiratory enteroviruses have not been well characterized in the East African region. We sought to molecularly characterize HEV-68 isolated in Kenya in 2008 to 2010 in order to understand their genetic diversity. A total of six (6) isolates were analyzed. Viral RNA was extracted followed by RT-PCR amplification of VP1 capsid protein coding gene. PCR amplicons were sequenced and the resulting sequences compared to those of Fermon prototype strain and previously characterized strains from other countries. Pair-wise comparison of VP1 sequences of Kenyan HEV-68 isolates revealed 87.2-99.5% nucleotide identity. The Kenyan HEV-68 strains shared 86.0-88.4% and 91-99% nucleotide identities respectively, when compared to Fermon and previously characterized strains reported in Gen Bank. Multiple sequence alignment of VP1 sequences of Kenyan isolates with Fermon revealed 12 amino acid substitutions and one deletion in five of the isolates and 11 amino acid substitutions. Phylogenetic analyses revealed five of the Kenyan isolates clustered closely to HEV-68 strains which circulated in New York, USA and Yamagata, Japan; while only one clustered with those that circulated in the Netherlands, Europe. All the Kenyan isolates clustered away from Fermon indicating divergence from the prototype strain. These findings suggest HEV-68 strains isolated in Kenya during the period 2008 to 2010 were generally similar to those detected in other parts of the world. Majority of the Kenyan isolates were however more closely related to those detected in the United States and Japan. Surveillance and constant monitoring of HEV-68 is important in understanding their evolutionary dynamics.

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MICRORNAS AS BIOMARKER FOR ACTIVE TUBERCULOSIS INFECTION IN IMMUNOCOMPETENT AND IMMUNODEFICIENT

Grace W. Mwangoka[1], Paolo Miotto[2], Ilaria C. Valente[2], Luca Norbis[2], Giovanni Sotgiu[3], Klaus Reither[4], Norbert Heinrich[5], Francesco Aloi[6], Daniela M. Cirillo[2]

[1]Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania, [2]Emerging Bacterial Pathogens Unit, Division of Immunology, Transplantation and Infectious Diseases, S. Raffaele Scientific Institute, Milan, Italy, [3]Clinical Epidemiology and Medical Statistics Unit, Department of Biomedical Sciences, University of Sassari, Sassari, Italy, [4]Swiss Tropical and Public Health Institute, Basel, Switzerland, [5]Division for Infectious Diseases and Tropical Medicine, Ludwig Maxmillian University, Munich, Germany, [6]St. Francis, Nsambya Hospital, Kampala, Uganda

One of the most important priorities for tuberculosis control is the accurate diagnosis of individuals with active, infectious TB. This enables prompt treatment that both interrupts TB transmission and cures patients. Circulating nucleic acids (CNAs), including miRNAs present in serum, may serve as potential biomarkers for diagnosis and follow up in active and latent TB infection in immunocompetent and immunodeficient . The study enrolled consented participants, from 2 sites in Italy and 2 site in Africa (Tanzania and Uganda).Participants enrolled included healthy controls (HC), subjects with active TB (PTB), PTB with HIV and latent TB (LTBI). To minimize individual variation; sera from 10 participants from each category were pooled. miRNAs profile were measured using Taqman low density arrays qRT PCR. A Student's t test was used to compare mean concentrations of miRNAs with STATA 11. 672 miRNAs were analysed, 47 wmiRNAs were significantly up or down regulated and observed to be common in the active TB and HCs from both geographic region ($P<0.05$). 50 miRNAs were significantly expressed in active TB compared to LTBI; 37 and 11 miRNAs were up and down regulated respectively. Analysis performed following validation on single patients confirmed 4 common miRNAs from both the European and the African participants. Whereby: 7 miRNAs (3 European and 3 African specific and 4 common) were identified as discriminatory biomarkers for active TB disease. In conclusion, results from this study suggest that change in miRNAs expression levels plays a vital role in TB pathogenesis and could be biomarkers for TB diagnosis.

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PREVALENCE AND RISK FACTORS FOR LATENT TUBERCULOSIS INFECTION IN MILITARY PERSONNEL IN PERU

Giselle M. Soto[1], Cesar Munayco[2], Gabriela Soto[2], Moises Apolaya[3], Marianela Ore[4], Giovana Arenas[3], Jorge Fernandez[4], Juan Silvera[3], Antonio Tokumoto[3], Patricia Avila[4], Ruben Valle[1], Mariana Ramos[1], Daniel Ehlman[1], David Moore[5], Daniel G. Bausch[1]

[1]U.S. Naval Medical Research Unit - 6, Bellavista, Peru, [2]Dirección General de Epidemiología del Ministerio de Salud (DGE), Jesus Maria, Peru, [3]Dirección de Sanidad de la Fuerza Aerea del Perú (DISANFAP), Surco, Peru, [4]Comando de Salud del Ejército Peruano (COSALE), San Borja, Peru, [5]Laboratorio de Enfermedades Infecciosas, Universidad Peruana Cayetano Heredia, San Martin de Porres, Peru

Tuberculosis continues to be a global threat to public health. About of one-third of the world's population has latent tuberculosis infection (LTBI), with the highest rates generally in developing countries. Understanding the risk factors for LTBI is crucial for tuberculosis control. Populations in closed settings, such as military bases, are often at particularly high risk. Close contact with tuberculosis cases has been used to assess the risk of LTBI as well as active tuberculosis. Use of the tuberculin skin test (PPD) can identify persons with LTBI, but is often considered not useful to assess recent exposures in developing countries because it is assumed that the vast majority of persons are positive. We explored the prevalence and risk factors for LTBI in students and cadets in two military academies of the Peruvian Armed Forces. Participants were interviewed and received PPD placement. A total of 621 participants were enrolled, with a mean age of 19 years and 80% were male. Of 608 participants who returned for PPD evaluation, 118 (20%) were positive, with increasing prevalence with age; the multivariate logistic regression analysis showed that for every year of increased age the odds ratio for LTBI increased by 33%. Gender, area of birth and present residence, and close contact with tuberculosis cases or with relatives/friends with tuberculosis were not associated with LTBI. Despite the assumed high burden of tuberculosis in developing countries and closed settings, a minority of persons in our study were PPD positive and close contact with tuberculosis cases was not a risk factor for LTBI. However, increasing age was a good predictor for LTBI and should form the basis for targeted control efforts. We assume, but cannot be certain, that our study population is representative of the general population of Peru.

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INCIDENCE OF RESPIRATORY TRACT INFECTIONS AMONG PASTORALISTS BEFORE AND AFTER THE INTRODUCTION OF PCV-10 VACCINATION IN RURAL NORTHERN KENYA

Stephanie J. Hauck, Stephanie B. Gati, Bryan Grenfell

Princeton University, Princeton, NJ, United States

Worldwide, pneumonia is the top killer of children under five years old, taking the lives of 1.2 million children every year. Developing countries bear the highest burden of childhood mortality, and 30,000 of these yearly childhood pneumonia deaths occur in Kenya. To curb the effects of this killer disease, the Kenyan Ministry of Health introduced the 10-valent pneumococcal conjugate vaccine (PCV) to the routine immunization schedule in late 2010. Despite the introduction of PCV-10 to the schedule, however, inadequate access to vaccination in some parts of Kenya suggests immunization rates are too low to induce herd immunity in these communities. To explore the relationship between immunization for PCV-10 and respiratory infection rates, we conducted a retrospective study of

vaccination and outpatient records from two rural dispensaries who service pastoral populations in Laikipia county, Kenya. We found that PCV-10 coverage is very low, with only 33.3% of children receiving the first dose of PCV-10, 13.6% the second dose, and 6.15% the final dose. T-tests with unequal variance using Satterthwaite's degrees of freedom show no significant decrease in pneumonia incidence after vaccination began in Laikipia in March 2011 at either dispensary (Dispensary A: t (34.845) = 0.081, p = .468) and (Dispensary B: t (39.889) = 1.068, p = .146). However, the variance of pneumonia cases in months of high vaccination coverage is significantly lower than the variance of cases during low vaccination months (F (7,38) = 0.266, p = .037), suggesting that if great numbers of children are vaccinated with PCV-10, significant reductions in pneumococcal disease rates may occur.

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EVALUATION OF ACCEPTABILITY AND PERFORMANCE OF STOVE OPTIONS FOR REDUCING HOUSEHOLD AIR POLLUTION IN RURAL WEST KENYA

Nigel G. Bruce[1], Tamara Pilishvili[2], Bryan Christensen[2], Debbi Stanistreet[1], Jennifer Loo[2], Ibrahim Sadumeh[3], Justus Muoki[4], Fuyuen Yip[2], Lindsey Horton[2], Michelle Bashin[5], Mike Sage[2]

[1]University of Liverpool, Liverpool, United Kingdom, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Safe Water and Aids Project, Kisumu, Kenya, [4]University of Nairobi, Nairobi, Kenya, [5]Public Health Institute, Oakland, CA, United States

Relationships between household air pollution (HAP) and risk of key diseases suggest low levels are needed to realise most of the health benefit; furthermore, achieving low levels requires that households are willing to use effective stoves for all or most needs. This study aims to identify whether one or more solid fuel stoves are capable of both meeting user needs and delivering low HAP, and hence suitable for intervention studies and scaling up. The study was conducted in west Kenya using mixed methods. Candidate stoves were required to demonstrate ≥ 40% reduction in PM2.5 emissions in USEPA tests. A cooking demonstration assessed user views on those most suitable for local needs: the 6 best (2 rocket, 1 chimney rocket, 3 fan-assisted) were then evaluated in a cross-over design in 43 homes. Following baseline measurement of kitchen concentrations (CO, PM2.5), personal women (cook) and youngest child (< 5 yr) CO, and stove use with stove use monitors (SUMS, each home used one stove type for 2 weeks, with repeat assessment in the final 48 hrs. This cycle repeated until all homes used at least 5 stoves. Qualitative interviews at baseline and following use of each stove assessed user views and reasons for multiple stove use. Focus groups (FG) explored user views in comparing all stove types. Initial (Round 1&2) results for kitchen and personal HAP show reductions for all stove types, but not to the low levels sought. SUMS data show multiple stove use occurred, and high kerosene lamp emissions may also help explain post-intervention HAP levels. Qualitative findings indicate preference for the new stoves, women reporting smoke reduction and finding them cleaner, more fuel efficient and easy to use. However, a number of stove improvements are suggested which could reduce multiple stove use. For most women, stove cost is reported as a barrier, but the FGs identified ways these could be made more affordable and marketed. Full results (to be presented) will help guide technology development and adoption to help deliver substantive health benefits at scale.

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ANTIBIOTIC USE IN AN INFLUENZA-LIKE ILLNESS COHORT IN PERU, 2009-2011

Lizette O. Durand[1], Yeny Tinoco[1], Matthew R. Kasper[1], Eduardo Azziz-Baumgartner[2], Candice Romano[1], Hugo Razuri[1], Daniel G. Bausch[1]

[1]U.S. Naval Medical Research Unit - 6, Lima, Peru, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States

Influenza-like illness (ILI) affects 20% of the global population annually. Although over 95% of ILI cases are thought to be viral, several studies have shown that patients and physicians often confuse ILI with respiratory infections caused by bacteria, prompting antibiotic prescription and inducing antibiotic resistance. To date, there has been no study on the use of antibiotics in persons with ILI in Peru. Therefore, we collected data on medication use, both prescribed and over-the-counter, from a multi-site ILI cohort study in four distinct ecological regions of Peru during 2009-2011. We compared antibiotic use associated with region, gender, age, presence of co-morbidities, and use of other medications in the preceding 30 days by chi-square analyses performed in EpiInfo. Data were collected on 6,790 cases of ILI, of whom 53% were female, with a median age of 14.7 years (range newborn-108). Overall, 92% of study participants took some medication for their ILI, of which 13% were prescribed by a physician. Co-morbidities and previous medication use were reported in 15% and 24%, respectively. Virtually all of those who took prescribed medications also took over-the-counter ones. Antibiotics comprised 27% of all medications, of which 51% were prescribed and 62% were penicillin drugs. Interestingly, despite data showing that approximately 20% of ILI cases in the cohort are influenza, no person took an anti-influenza drug, although these are not readily available on the market or in private clinics in Peru. The proportion of antibiotic use was higher than all other drugs taken for ILI. Prescription drugs, including antibiotics, are clearly frequently taken by persons with ILI in Peru. Although the etiologic agent is unknown in the majority of cases, the results almost certainly demonstrate an overuse of antibiotics for ILI, despite universal recommendations against the use of antibiotics for this syndrome. Increased availability of on-site diagnostics and dissemination of guidelines on the management of ILI at healthcare centers could improve this situation.

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XPERT MTB/RIF FOR THE DIAGNOSIS OF TUBERCULOSIS IN CHILDREN - A SYSTEMATIC REVIEW AND META-ANALYSIS

Anne K. Detjen[1], **Andrew R. DiNardo**[2], Jacinta Leyden[3], Karen R. Steingart[4], Dick Menzies[5], Ian Schiller[6], Nandini Dendukuri[7], Anna M. Mandalakas[2]

[1]The International Union against Tuberculosis and Lung Disease, New York, NY, United States, [2]Baylor College of Medicine, Houston, TX, United States, [3]Rice University, Houston, TX, United States, [4]Cochrane Infectious Diseases Group, Liverpool School of Tropical Medicine, United Kingdom, [5]Respiratory and Epidemiology Clinical Research Unit, Montreal Chest Institute, McGill University, Montreal, QC, Canada, [6]Division of Clinical Epidemiology, McGill University Health Centre – Research Institute, Montreal, QC, Canada, [7]Division of Clinical Epidemiology, McGill University, Montreal, QC, Canada

In 2011, the WHO recommended Xpert MTB/RIF for the diagnosis of tuberculosis (TB) and MDR TB in all age groups despite a lack of pediatric data at that time. We conducted a systematic review to assess the diagnostic accuracy of Xpert MTB/RIF for pulmonary TB (PTB) in children. We performed database searches for relevant studies in all languages through April 2013. We included randomized-controlled, cross-sectional, and cohort studies involving children (< 15 years) with presumed TB. We extracted data separately for expectorated sputum (ES), induced sputum (IS), nasopharyngeal aspirates (NPA), and gastric aspirates (GA). We performed meta-analysis to determine pooled sensitivity and specificity. We included 10 studies in PTB. Five studies (41.7%) were conducted in

low or lower middle-income countries. Against a reference standard of culture, pooled sensitivities were 69% (95% Credible Interval 55-81) for ES and IS combined (7 studies) and 75% (59-90) for GA (5 studies). In HIV-infected children, sensitivity was 77% (60-89) in ES/IS versus 59% (44-72) in HIV-uninfected children. Sensitivity in ES/IS in children aged 0-4 was 57% (36-74) versus 83% (68-92) in children aged 5-15. Pooled specificity was >95% in all subgroups assessed using culture as a reference standard. In children with smear positive disease, pooled sensitivity was 96% (90-99) for ES and IS and 95% (83-99) for GLA. Pooled sensitivity in smear negative disease was 76% (58-90) for ES/IS and 78% (59-92) for GLA. As Xpert MTB/RIF is being rolled out in TB high burden settings it becomes available for children as an alternative to smear microscopy. Xpert MTB/RIF is highly specific for TB in children. However, sensitivity estimates are estimated with poor precision due to the sparse data available. There is a greater need for pediatric studies of Xpert to support guidelines for use in this population.

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COMPARISON OF NASOPHARYNGEAL SWABS COLLECTED FOR PNEUMOCOCCAL COLONIZATION AND NASAL SWABS IN THE IDENTIFICATION OF VIRAL RESPIRATORY INFECTIONS IN PERU

Carlos Grijalva[1], Philip Budge[1], Marie Griffin[1], Ana Gil[2], Kathryn Edwards[1], Hector Verastegui[2], Monika Johnson[1], Stella Hartinger[2], Claudio Lanata[2], John Williams[1]

[1]Vanderbilt University School of Medicine, Nashville, TN, United States, [2]Instituto de Investigacion Nutricional, Lima, Peru

We sought to determine agreement in detection of respiratory viruses using RT-PCR testing between two different types of samples collected on the same day: nasal swabs preserved in viral transport medium (NS) and nasopharyngeal swabs preserved in skim milk-tryptone-glucose-glycerol [STGG] media (NP). Samples were collected as part of a prospective household-based cohort study of Andean children aged less than 3 years. Nasal swabs were collected during episodes of acute respiratory illness for identification of respiratory viruses including influenza, human metapneumovirus (MPV), respiratory syncytial virus (RSV), human rhinovirus (HRV), parainfluenza virus 3 (PIV) and adenovirus (AdV). NS used a Dacron swab placed into each nostril sequentially, rotated beneath the turbinates, and placed into viral transport medium, which was then aliquoted into lysis buffer and stored at -80C. NP swabs were collected on a monthly basis to study colonization with *Streptococcus pneumoniae*. NP used a Rayon wire-handled swab placed through one nostril into the posterior nasopharynx, rotated for 5 seconds, placed into STGG and stored at -80C. A random sample of paired NP and NS samples collected from the same child on the same day was selected. Nucleic acid was extracted and tested for respiratory viruses by real-time monoplex RT-PCR. We evaluated the agreement between NP and NS samples in viral detection using the kappa coefficient and compared viral loads in NP and NS samples using RT-PCR cycle thresholds (CT). We studied 260 paired NP and NS samples. The kappa coefficient between NP and NS virus testing results was 0.70 (AdV); 0.87 (RSV); 0.88 (influenza); 0.92 (PIV3); 0.96 (HRV); and 0.97 (MPV). Median CT values were not statistically different between NP and NS samples across most respiratory viruses, except for influenza and RSV for which CTs were slightly lower in NP than in NS (all $p<0.05$). The agreement between NS and NP samples was very high, indicating NP samples could be used as a single, efficient collection strategy for field studies of both respiratory viruses and bacteria.

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LOPHOMONAS SP. IN RESPIRATORY TRACT SECRETIONS IN HOSPITALIZED CHILDREN WITH PNEUMONIA AND *BORDETELLA PERTUSSIS* CO-INFECTION

Rito Zerpa[1], Elsa Ore[2], Carmen Quispe[2], Lilian Patiño[2], Margarita Alvarado[2], Paolo A. Wong[1]

[1]Instituto de Medicina Tropical Daniel A. Carrión, Universidad Nacional Mayor de San Marcos, Lima, Peru, [2]Instituto Nacional de Salud del Niño, Perú, Lima, Peru

Lophomonas sp. is a protozoan that is found in the digestive tract of cockroachs: *Periplaneta americana* and *Blatta germanica*. There are few reports of this emerging protozoan infection in humans, mainly affecting the lower respiratory tract in patients with severe lung disease. *Lophomonas sp.* has recently been reported in patients with asthma, as well as the discovery of protozoa in the respiratory tract of children with pneumonia. The aim of the study was to investigate *Lophomonas* in respiratory samples of children with pneumonia and in patients with a clinical diagnosis of pertussis, treated at the National Institute of Child Health, national reference center for pediatric diseases in Lima, Peru, in the period January to December 2012 and from January to March 2013. 558 samples were worked: 471 from tracheal aspirate, 40 from bronchoalveolar lavage and 47 from nasopharyngeal aspirate. This last group corresponding to children with a clinical diagnosis of pertussis. *Lophomonas* was found in 17/558 (3.04%) samples of children with pneumonia, six of them were diagnosed with pertussis. A sample with *Lophomonas sp.* and *Bordetella pertussis* coinfection was found out. In conclusion, it is necessary to search for *Lophomonas sp.*, emerging protozoan upper and lower respiratory infections, mainly in children with pneumonia and in patients diagnosed with pertussis.

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ESTABLISHMENT AND SUCCESSES OF THE UGANDA NATIONAL VIRAL HEMORRHAGIC FEVER SURVEILLANCE PROGRAM AND HIGH-CONTAINMENT LABORATORY, 2010-2013

Trevor Shoemaker[1], Stephen Balinandi[1], Alex Tumusiime[1], Joseph Wamala[2], Barbara Knust[3], Ilana Schafer[3], Shelly Campbell[3], Debi Cannon[3], Bobbie Rae Erickson[3], Arideth Gibbons[3], Luke Nayakarahuka[4], Julius Lutwama[4], Edward Mbidde[4], Ute Ströher[3], Pierre Rollin[3], Stuart Nichol[3]

[1]Centers for Disease Control and Prevention-Uganda, Entebbe, Uganda, [2]Ministry of Health, Kampala, Uganda, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States, [4]Uganda Virus Research Institute, Entebbe, Uganda

Uganda is endemic for viral hemorrhagic fevers (VHF) and other zoonotic diseases. In July 2010 the Viral Special Pathogens Branch, CDC, the Uganda Virus Research Institute (UVRI), and the Ministry of Health established a first of its kind National VHF surveillance program. In addition, a permanent high-containment laboratory was established at UVRI. This lab serves as the national VHF reference laboratory, and an East Africa regional resource. The laboratory can perform real-time PCR, IgM, IgG and antigen capture ELISA for Ebola, Marburg, Rift Valley fever, and Crimean-Congo hemorrhagic fever viruses. To date, suspect VHF samples from over 20 districts in Uganda, and 5 East and Central African countries, have been sent for rule-out testing. The program has tested over 2000 samples from human surveillance, serosurveys, primates, livestock, and VHF outbreaks. 1364 samples have been tested for surveillance and outbreak activities, resulting in confirmation of 5 independent filovirus outbreaks. Four Ebola outbreaks have occurred: two in Luwero District (2011, CFR=100%; 2012, CFR=57%; both Sudan virus), one in Kibaale District (2012, CFR=71%; Sudan virus), and one in Isiro, DRC (2012, CFR=54%; Bundibugyo virus). One Marburg outbreak occurred in Kabale, Kamwenge, and Ibanda districts in 2012 (CFR=58%.). Testing was completed for a national Uganda serosurvey of 587 human blood

samples looking for evidence of past infection with Ebola, Marburg, RVF, and CCHF. The program has also tested 244 primate and livestock samples for VHFs, including 204 samples from the Karamoja region where 35% were positive by IgG for CCHF, showing evidence of actively circulating CCHF virus in Uganda. The successes of this program show how having a functional, comprehensive, and timely VHF surveillance system in Uganda, and East Africa, greatly contributes to limiting the extent of outbreaks through early detection and response. This program also advances the knowledge of other high-hazard pathogens of international concern in Uganda, and the region, and should serve as a model for further expansion throughout Africa.

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RE-EMERGENCE OF BUNDIBUGYO VIRUS AFTER A FIVE YEAR HIATUS -- ISIRO, THE DEMOCRATIC REPUBLIC OF THE CONGO, 2012

Ilana J. Schafer[1], Andrea M. McCollum[1], Barbara Knust[1], Jean-Jacques Muyembe[2], Robert Shongo[3], Benoit Kebela[3], Kiyele Musa[3], Eric Bergeron[1], Christina Spiropoulou[1], Brian Bird[1], Elisabeth Pukuta[2], Gary Kobinger[4], Félix Adima[5], Claudine Nseye[3], Mathias Mossoko[3], Félix Mulangu[3], Emmanuel Lampaert[6], Carolina Nanclares[7], Deborah Cannon[1], Steven Balinandi[8], Alex Tumusiime[8], Olimpia de la Rosa[7], Miriam Alia[7], Bobbie Rae Erickson[1], Trevor Shoemaker[8], Ute Ströher[1], Pierre E. Rollin[1], Stuart Nichol[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Institut National pour la Recherche Biomédicale, Kinshasa, Democratic Republic of the Congo, [3]Ministry of Health, Kinshasa, Democratic Republic of the Congo, [4]Public Health Agency of Canada, Winnipeg, MB, Canada, [5]Haut-Uélé District Health Department, Isiro, Democratic Republic of the Congo, [6]Médecins sans Frontières, Kinshasa, Democratic Republic of the Congo, [7]Médecins sans Frontières, Barcelona, Spain, [8]Centers for Disease Control and Prevention, Kampala, Uganda

On August 16, 2012, two patients in Isiro Health Zone, The Democratic Republic of Congo (DRC) tested positive for Bundibugyo virus (BDBV), identifying the second Ebola Hemorrhagic Fever (EHF) outbreak attributed to BDBV – the first occurring in Uganda in 2007. An international response was immediately launched to control the outbreak. Patient epidemiologic and clinical data were collected via a standardized case report form. A field laboratory tested blood samples by RT-PCR, and serology diagnostics were performed in Uganda. Cases were classified as suspect (clinical criteria), probable (suspect plus epidemiologic criteria), or laboratory confirmed. Bivariate data analysis was used to evaluate cases and non-cases for predictors of BDBV infection, and cases for predictors of death. A total of 36 confirmed, 16 probable, and 7 suspect EHF cases were identified, with 133 initially suspected cases ruled out. Among the 52 confirmed and probable cases, the case fatality rate (CFR) was 53.8%, age range was 0 – 70 years (median=40 years), 76.9% were female, and 25.0% were healthcare workers. Among all patients evaluated for EHF, factors significantly associated ($p<0.05$) with being a case were female gender, fever, vomiting, diarrhea, fatigue, conjunctivitis, difficulty swallowing, difficulty breathing, hiccups, and anorexia. Patients with a cough were significantly less likely to be a case. Among EHF cases, symptoms significantly associated with death were hemorrhagic signs, cough, difficulty swallowing, difficulty breathing, conjunctivitis, and hiccups. Five unlinked chains of virus transmission were identified, indicating that EHF cases remained unidentified. June 1, 2012 was the earliest discovered case onset. Like the 2007 outbreak, this BDVB outbreak had a lower CFR than is seen with *Zaire ebolavirus* and *Sudan ebolavirus* species. The last confirmed case was isolated 57 days after outbreak detection. Prompt local and international efforts in field laboratory establishment, case finding, and case isolation were crucial to the successful containment of the outbreak.

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ROTAVIRUS INFECTION IN CHILDREN IN A RURAL COMMUNITY IN PISCO, PERU

Carlos A. Figueroa, Maria E. Silva, Giannina Luna, Claudio Rocha, Drake Tilley, Daniel G. Bausch

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

Rotavirus is the leading cause of severe diarrhea in children under 5 years of age worldwide, causing up to 50% of childhood hospitalizations for diarrhea in industrialized countries, and an even higher proportion in developing countries. However, data on rotavirus transmission rates in the community are much more sparse, especially in developing countries. Community-based data will be vital in assessing the effectiveness of rotavirus vaccination as the vaccine becomes more widely employed. We used data and samples available from a study on water quality to explore the prevalence of rotavirus infection in rural communities outside the town of Pisco, Peru. The study was conducted in 2010, one year after rotavirus vaccination was introduced in Peru's national immunization program, with reported rotavirus vaccine coverage in Ica region at the time of 64%. A convenience sample of 192 houses was selected and stool samples taken from one child age ≤ 5 years old from each household. Stools were tested for rotavirus by real time RT-PCR according to CDC guidelines. Of the 192 children enrolled, 54 (28%) were rotavirus infected. The proportion of rotavirus-infected children did not differ significantly between children who did and did not report an episode of diarrhea in the preceding two weeks: 11/32 (33%) and 43/160 (27%), respectively. The median age of the rotavirus-positive children was 24.5 months (range 5-48 months) and 50% were male. Unfortunately, because the original aim of the study was not oriented toward rotavirus, no specific rotavirus vaccination history on each child was taken. The finding of frequent rotavirus infection in children who did not recently suffer diarrhea suggests that additional factors, such as infectious dose, underlying co-infections or morbidities, or genetic predisposition are involved in producing clinical disease due to rotavirus infection. The results also provide baseline data useful for future assessment of rotavirus vaccine effectiveness in Peru.

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NIAKHA VIRUS: A NOVEL MEMBER OF THE FAMILY *RHABDOVIRIDAE* ISOLATED FROM PHLEBOTOMINE SANDFLIES IN SENEGAL

Nikos Vasilakis[1], Steven Widen[1], Sandra V. Mayer[1], Robert Seymour[1], Thomas G. Wood[1], Vsevolod Popov[1], Hilda Guzman[1], Amelia P. Travassos da Rosa[1], Elodie Ghedin[2], Edward C. Holmes[3], Peter J. Walker[4], Robert B. Tesh[1]

[1]University of Texas Medical Branch Health, Galveston, TX, United States, [2]University of Pittsburgh, Pittsburgh, PA, United States, [3]The University of Sydney, Sydney, Australia, [4]CSIRO, Geelong, Australia

Members of the family *Rhabdoviridae* have been assigned into eight genera but many remain unassigned. Rhabdoviruses have a diverse host range that includes terrestrial and marine animals, invertebrates and plants. Transmission requires arthropod vectors such as mosquitoes, midges, sandflies, ticks, aphids and leafhoppers, in which they replicate. Here we characterize Niakha virus (NIAV), a previously uncharacterized rhabdovirus isolated from phebotomine sandflies in Senegal. Analysis of the 11,124 nt genome sequence indicates that it encodes the five common rhabdovirus proteins with alternative ORFs in the M, G and L genes. Phylogenetic analysis of the L protein indicate that NIAV's closest relative is Oak Vale rhabdovirus, although still so phylogenetically distinct that it may be not classified as a member of the eight recognized *Rhabdoviridae* genera. This observation highlights the vast, and yet not fully recognized diversity, of this family, some members of which could potentially jump species boundaries in the future.

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EASTERN EQUINE ENCEPHALITIS VIRUS: REEMERGENCE AND EXPANSION IN THE NORTHEASTERN UNITED STATES

Theodore G. Andreadis, Philip M. Armstrong, Goudarz Molaei

Center for Vector Biology and Zoonotic Diseases, The Connecticut Agricultural Experiment Station, New Haven, CT, United States

Eastern equine encephalitis (EEE) virus is the most deadly mosquito-borne pathogen in North America with an estimated human case fatality rate of 35 to 75%. EEE virus activity is most common in and around freshwater hardwood swamps in the Atlantic and Gulf Coast states and in the Great Lakes region, where the primary mosquito vector *Culiseta melanura* resides. Since the discovery of EEE virus in the 1930s, outbreaks in temperate regions have been sporadic, both temporally and spatially, highly focal, and largely unpredictable. However, over the last decade, we have witnessed a sustained resurgence and change in dynamics of EEE virus activity within long-standing foci in the northeastern U.S. and unprecedented northward expansion into new regions where the virus had been historically rare or previously unknown, including northern New England and eastern Canada. This has resulted in severe disease in humans (46 cases with 16 fatalities) and domestic animals (173 cases). The factors responsible for reemergence of EEE virus are largely unknown but are likely complex reflecting ongoing changes in the ecology and epidemiology of this virus. Long-term changes in land-use, including wetlands restoration and suburban development, and increases in human population density near critical habitats may be important components. Weather conditions associated with climate change are also likely to be contributing factors. These include mild winters, hot summers and extremes in both precipitation and drought that increase vector abundance and distribution, elongate the virus transmission season, and increase the intensity of virus transmission by increasing the frequency of blood feeding and rate of virus replication in mosquitoes. These and other underlying factors associated with the introduction, amplification, persistence, and range expansion of EEE virus in the region including: 1) vector mosquito abundance and distribution that drive viral amplification and spillover into human and equine populations, 2) species-specific mosquito-avian interactions that favor amplification, 3) virus titers in primary and secondary mosquito vectors, and 4) genetic variation in regional EEE virus strains that provide evidence for local overwintering, evolution and extinction of EEE virus strains, with periodic reintroduction from southern sources, will be examined.

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EMERGING PATHOGENS IN MULTIPLE BAT SPECIES IN MADRE DE DIOS, PERU: *LEPTOSPIRA* AND PARAMYXOVIRUSES

Karen Segovia[1], Bruno M. Ghersi[2], Maria E. Silva[2], Gabriela Salmon-Mulanovich[2], Enrique Canal[2], Hugo Razuri[2], Victor Pacheco[3], Matthew R. Kasper[2], Joel M. Montgomery[2], Daniel G. Bausch[2]

[1]San Marcos University School of Veterinary Medicine, San Borja, Peru, [2]U.S. Naval Medical Research Unit - 6, Lima, Peru, [3]Natural History Museum, Universidad Nacional Mayor de San Marcos, Lima, Peru

In recent years, bats have attracted considerable attention as hosts of emerging and other pathogens relevant to public health. We trapped bats and harvested their tissues for analysis near seven communities in the Madre de Dios Region in the southern Amazon basin of Peru as part of a study to explore the impact of anthropogenic habitat perturbation in the region (the building of the Peruvian interoceanic highway) on the distribution of reservoirs and pathogens. Bat kidneys were tested for *Leptospira* by PCR using primers that amplify 16S rRNA. Spleens were tested for paramyxovirus by nested PCR targeting the conserved motifs of the polymerase pol gene. A total of 432 bats from 24 different genera were captured, of which 32 (7%) were positive for *Leptospira*. All positive bats belonged to one of nine genera of the family *Phyllostomidae*, including the genera *Trachops* and *Lophostoma*. Twenty-six (81%) of the *Leptospira* positive bats were adults, while age could not be determined in the remaining 6 (19%). Infected animals were identified in 6 of the 7 sites sampled. Sequencing of PCR products is underway to identify the specific species of *Leptospira* implicated. Paramyxovirus testing was performed on 263 bats, of which 3 (1%) were positive. All 3 positive bats were adults of the *Sturnira lilium* species collected in one location in Iberia District. Sequence analysis placed the paramyxoviruses in the Avulavirus or Rubulavirus genera. Avulaviruses are known to date only to infect birds, while rubulaviruses such as Tioman, Mapuera, and Menangle have been described in fruit- and insect-eating bats, making Rubulavirus the more likely genus implicated here. Of note, rubulaviruses have been associated with encephalitis and influenza-like illness in humans. This is the first report of *Leptospira* infection in *Trachops* and *Lophostoma* in Peru, as well as of paramyxovirus infection in any bat in Peru, expanding our understating of the host and geographic range of these potentially emerging pathogens. Testing for other pathogens, including coronaviruses, is underway.

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AGE-STRATIFIED SEROLOGICAL SURVEY OF SYLVATIC CHIKUNGUNYA VIRUS IN NONHUMAN PRIMATES IN SENEGAL

Benjamin M. Althouse[1], Mathilde Guerbois[2], Amadou A. Sall[3], Mawlouth Diallo[3], Diawo Diallo[3], Ousmane Diop[3], Brenda Benefit[4], Evan Simons[4], Douglas M. Watts[5], Scott C. Weaver[2], Kathryn A. Hanley[4], Derek A. Cummings[6]

[1]Santa Fe Institute, Santa Fe, NM, United States, [2]University of Texas Medical Branch, Galveston, TX, United States, [3]Institut Pasteur, Dakar, Senegal, [4]New Mexico State University, Las Cruces, NM, United States, [5]University of Texas at El Paso, El Paso, TX, United States, [6]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Sylvatic chikungunya virus (CHIKV) has been isolated in Senegal over the past 50 years. Until recently, virus isolation was predominantly from mosquito collections, and the virus was only isolated from nonhuman primates (NHP) after opportunistic capture. Here we present an age-stratified serological survey of CHIKV in three NHP species in Senegal and calculate forces of CHIKV infection for each species. African green monkeys (*Chlorocebus sabaeus*), patas monkeys (*Erythrocebus patas*), and Guinea baboons (*Papio papio*) were collected in the dry season in each of three years (2010-2012) from in Kedougou, Senegal. Primates were trapped, sedated, and bled and sera were tested for IgM by ELISA and IgG by PRNT. Ages of primates were quantified using pattern of tooth eruption and wear determined from photographs and dental casts; weight and other anthropometric measurements were also taken. Force of CHIKV infection was calculated using catalytic models with bootstrap confidence intervals. Random effect logistic models were fit to find associations between age, month of collection, and species with seropositivity. A total of 219 African green monkeys, 78 patas, and 440 baboons were collected between 2010 and 2012. Across all years, 66%, 36%, and 73% were seropositive for CHIKV antibody by PRNT50, respectively. Forces of infection were high, ranging from 0.13 per year (95% Confidence Interval [CI]: 0.07, 0.21) for patas in 2012 to 1.15 per year (95% CI: 0.81, 3.83) for African green monkeys in 2010. Logistic models with random effects for troop indicated age as significantly positively associated with PRNT50 positivity (Odds ratio [OR]: 1.030 (95% CI: 1.023, 1.036)), and significantly less positivity in patas compared to African green monkeys (OR: 0.73, (95% CI: 0.60, 0.89)). To our knowledge this is the first study of CHIKV transmission dynamics in its sylvatic reservoir host. It reveals very high forces of infection of CHIKV for all NHP species tested, with rates of seropositivity approaching 100% as primate age increases. Our demonstration of a CHIKV reservoir carries important consequences for individuals living or working in proximity to primate populations in Senegal, where CHIKV has the potential to cause major morbidity.

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HERPES SIMPLEX AS THE MOST COMMON CAUSE OF ENCEPHALITIS IN PERU

Nicanor Mori[1], Silvia Montano[1], Ada Romero[1], **Mark P. Simons**[1], Drake Hamilton Tilley[1], Joseph Zunt[2]

[1]U.S. Naval Medical Research Unit - 6, Callao, Peru, [2]University of Washington, Seattle, WA, United States

Herpes simplex encephalitis (HSE) causes significant morbidity and mortality within developing countries, where the ability to diagnosis and treat HSE is limited. Our aim was to describe the clinical and cerebrospinal fluid (CSF) characteristics of HSE in patients presenting with symptoms of encephalitis to a network of hospitals in Peru. This was a prospective study of patients aged 28 days or older presenting with symptoms of encephalitis at nine Peruvian hospitals in three different geographical regions (coast, mountains and jungle) between February 2009 and March 2012. We enrolled patients presenting with clinical symptoms of encephalitis and diagnosis of HSE was confirmed by detection of HSV DNA in CSF using PCR. In this study 223 patients met clinical criteria for encephalitis and were included in the final analysis. Mean age of the patients was 5.41 years for children and 41.8 years for adults. 66.7% of children and 47.6% of adults were male. The mean time from onset of symptoms to hospital presentation was 9 days (range: 1 - 31). Headache, fever, seizures, neck stiffness, dystaxia, and nausea were the most common clinical symptoms. Seizures were more frequent in children (p=0.005), while headaches and neck stiffness were more frequent in adults (p=0.013 and 0.048, respectively). CSF was normal in 5.5% patients with abnormal glucose seen in 55.6%. Leukocyte counts, predominantly lymphocytes, were higher in adults than in children (p=0.031). HSV as determined by PCR was the etiology in 36 (16.1%) patients (21 adults ,15 children). The majority of HSE (88.9%) was due to HSV-1. HSV-2 was found in 2 patients from each age group. Co-infections with HIV were found in 5 (13.8%) adults and 3 patients also had *Cryptococcus neoformans* meningitis. HSV-1 was found to be the most common cause of encephalitis in Peru and emphasizes the need for improvements in diagnostic capabilities and acyclovir availability in developing countries.

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GENETIC CHARACTERIZATION OF NOROVIRUSES AMONG PERUVIAN ARMY RECRUITS IN THE AMAZON BASIN

Sarah B. Ballard[1], C. Giannina Luna[2], Sonia Apaza[3], Susan Espetia[3], Mayuko Saito[4], Maria E. Silva[2], Erik J. Reaves[2], Claudio Rocha[2], Karla J. Vilela[2], Drake H. Tilley[2], David L. Blazes[5], Robert H. Gilman[1], Daniel G. Bausch[6]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]U.S. Naval Medical Research Unit - 6, Lima, Peru, [3]Laboratorio de Investigacion y Desarrollo (LID), Universidad Peruana Cayetano Heredia, Lima, Peru, [4]University of California San Diego School of Medicine, San Diego, CA, United States, [5]Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [6]Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States

Norovirus is the number one cause of acute gastroenteritis worldwide, afflicting 21 million Americans and killing 200,000 children under five in the developing world each year. Understanding norovirus genetic variation may be important in the development of an effective vaccine. We assessed the various genotypes of norovirus circulating in a cohort of Peruvian military recruits under active surveillance for acute gastroenteritis between 2005-2011 in Iquitos, Peru. Stool specimens were collected from randomly selected participants, 200 with acute gastroenteritis and 200 healthy controls, and tested for norovirus genogroups GI and GII by real-time RT-PCR. Positive samples were genotyped by sequencing the C region of the capsid gene. Sequence fragments were aligned and compared to norovirus sequences available in the GenBank database. Norovirus was detected in 40/360 (11.1%) samples, 26/184 (14.1%) cases and 14/176 (8.0%) controls (the epidemiologic and clinical significance of these findings are discussed in a companion abstract). Of the 25 noroviruses that could be genotyped (18 from cases and 7 from controls), 11 were GI and 14 were GII. The predominant GI genotype was GI.4 (7 persons), followed by one each of GI.1, GI.3, GI.5, and GI.7. The predominant GII genotype was GII.4 (6 persons), followed by GII.17 (2 persons) and one each of GII.5, GII.6, GII.14, GII.15, and GII.16. Of the four GII.4 positive cases that could be further differentiated by variant, all were GII.4 Den Haag (2006b). The sample size was too small for meaningful statistical analysis, but the GII.4 genotype was the most prevalent genotype identified in cases, and it was not identified in controls. Multiple norovirus variants circulated in both cases and controls in this study, without other obvious associations with pathogenicity. Further research is needed to explore the possible clinical significance of the numerous variants of norovirus and to guide vaccine development.

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RODENT SPECIES AND THEIR CORRELATION WITH HUMAN SEROPOSITIVITY FOR ZOONOTIC INFECTIONS IN GHANA

Shirley C. Nimo Paintsil[1], Elisabeth Fichet-Calvet[2], Emad Mohareb[3], Maria Morales[3], Joseph H. Bonney[1], Kwesi Obiri-Danso[4], William K. Ampofo[1], Randal J. Schoepp[5], Karl C. Kronmann[6]

[1]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [2]Bernhard-Nocht Institute for Tropical Medicine, Hamburg, Germany, [3]U.S. Naval Medical Research Unit - 3, Cairo, Egypt, [4]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [5]United States Army Medical Research Institute of Infectious Disease, Fort Detrick, MD, United States, [6]Naval Medical Center, Portsmouth, VA, United States

Rodents serve as reservoirs and/or vectors for several human infections which account for high morbidity and mortality in Africa. The remarkable expansion of human population has brought them into increasing contact with these mammals, thereby disrupting their habitats and increasing opportunities for disease transmission. To investigate possible risk factors for exposure to some of these pathogens, 764 small mammals were collected from ten communities in Ghana together with 657 human sera from healthy adults living in the same communities. Rodents were captured by setting Sherman collapsible traps along marked lines in fields (outdoors) and houses (indoors) totaling 9,269 night traps for three consecutive nights. The small mammals caught constituted ten genera of which whole blood of two rodents (0.3%) *Mus (Nannomys) sp.* tested positive for arenaviruses and one kidney tissue from Croccidura sp tested positive for *Leptospira* by conventional polymerase chain reaction (PCR). All rodent lung tissues were negative for Hantaviruses (Dobrava and Puumala serotypes). Using an in-house enzyme-immunoassay (ELISA), human serum showed evidence of arenavirus antibodies in 34 samples (5%). Antibodies to Puumala and Dobrava serotypes and Leptospirosis were also detected in 11%, 12% and 21% respectively with commercial kits. The occurrence of immunoglobulin G (IgG) antibodies to Dobrava and Puumala serotypes was more common in females (54%) than in males whereas the opposite was observed for Lassa virus (LASV) and Leptospirosis (52%). Human exposure to zoonotic infections was observed to cut across all age groups. Seropositivity was highest for anti-LASV at site 7 (29%), anti-hantavirus (Dobrava serotype) at site 10 (26%), and anti-*Leptospira* at site 8 (19%) located in the Eastern, Brong Ahafo, and Northern Regions respectively. Fifty six individuals had been exposed to more than one of the rodent-borne infections tested whereas 208 had been exposed to only one type of infection. The known reservoirs of the different pathogens that were tested in the human sera were captured in most of the study sites but human exposure could not be linked to their presence. This study suggests that 40% of residents in rural farming communities in Ghana have measurable antibodies to at least one rodent-borne disease (LASV, hantavirus, or Leptospirosis), which is not surprising given the ubiquitous presence of rodents in subsistence farming communities.

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PHYLODYNAMIC AND PHYLOGEOGRAPHIC PATTERNS OF EASTERN EQUINE ENCEPHALITIS VIRUS IN THE NEW WORLD

Albert J. Auguste[1], Naomi L. Forrester[1], David Wentworth[2], Rebecca A. Halpin[2], Philip M. Armstrong[3], Thomas R. Unnasch[4], Grace Leal[1], Scott C. Weaver[1]

[1]The University of Texas Medical Branch, Galveston, TX, United States, [2]J. Craig Venter Institute, Rockville, MD, United States, [3]Center for Vector Biology and Zoonotic Diseases, The Connecticut Agricultural Experiment Station, New Haven, CT, United States, [4]University of South Florida, Tampa, FL, United States

Eastern equine encephalitis virus (EEEV) is a mosquito-borne alphavirus (Family; Togaviridae) of significant public and veterinary health importance throughout the Americas. EEEV exists as one antigenic complex but can be further classified into four lineages or subtypes based on serologic and phylogenetic analyses. Lineage I primarily consists of North American and Caribbean isolates, and lineages II-IV, which consist of Central and South America isolates, has been proposed to comprise a distinct species. Although EEEV is largely maintained locally (*in situ*), there is evidence of gene flow among and within countries. Lineage I strains utilize different hosts and vectors, and possess distinct neurovirulence characteristics, which are typically not observed among lineage II-IV isolates. This study aims to characterize EEEV genetic diversity, describe its molecular epidemiology, identify genetic determinants of EEEV emergence and virulence, and infer the phylodynamic and phylogeographic histories of EEEV. To this end, we performed a Bayesian analysis of EEEV complete genomes derived using next-generation sequencing. Results suggest differences in selective constraints and substitution rates among EEEV lineages. The data also suggests a Peruvian origin for EEEV, and that the virus spread to north eastern US prior to its expansion into other regions of the US. There is also evidence of significant gene flow within North America, suggesting that state level control measures would be inadequate for local elimination of the virus.

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EVASION OF HOST IMMUNE RESPONSE BY SEVERE FEVER WITH *THROMBOCYTOPENIA* SYNDROME VIRUS (SFTSV)

Félix W. Santiago[1], Lina Covaleda[1], Maria Sanchez[2], Ana Diaz-Virreta[1], Xue-jie Yu[1], Adolfo Garcia-Sastre[2], Patricia Aguilar[1]

[1]University of Texas Medical Branch, Galveston, TX, United States, [2]Mount Sinai School of Medicine, New York, NY, United States

Severe Fever with *Thrombocytopenia* Syndrome virus (SFTSV) is a novel member of the family Bunyaviridae, genus Phlebovirus. This virus was recently isolated from patients suffering from fever, thrombocytopenia, and hemorrhagic manifestations. SFTSV displays a mortality rate of 12% to 30% and direct human-to-human transmission has been reported. Due to the recent emergence of this pathogen, limited knowledge is available about the mechanism(s) involved in disease pathogenesis and the molecular mechanism(s) by which SFTSV suppresses innate immune responses. The type I interferon (IFN) responses are crucial for the development of antiviral immunity and therefore many pathogens have developed strategies that subvert these responses by blocking production of IFN or blocking IFN signaling. Indeed, type I IFN suppression has been described in other members of the genus. Likewise, we have observed that SFTSV infection inhibits type I IFN responses. SFTSV infection triggers the formation of cytoplasmic vesicles in which key components of the Type I IFN response, such as the cytosolic viral RNA receptor retinoic acid-inducible gene 1 (RIG-I) and its regulator the E3 ubiquitin ligase TRIM25, co-localize with viral proteins. Interestingly, the expression of the SFTSV nonstructural protein (NSs) is sufficient for the formation of vesicles and the co-localization of RIG-I and TRIM25 within them. SFTSV NSs not only co-localizes but also interacts with RIG-I and TRIM25. Furthermore, NSs inhibits the activation of the IFN-β promoter induced by virus infection or double-stranded RNA (dsRNA). Taken together, these data suggest that NSs inhibits type I IFN-mediated host protective innate immunity against viral infection by "sequestering" RIG-I and TRIM25 into the NSs-induced vesicles. Our studies provide mechanistic insights into viral pathogenesis and define a novel immune evasion strategy for subversion of host innate immune responses. This information will provide new targets for preventive and therapeutic interventions against SFTSV and other related pathogenic RNA viruses.

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NOROVIRUS GASTROENTERITIS AT A SKILLED NURSING FACILITY

Abimbola Ogundimu[1], Mosunmola Lizzy Adeyemi[2]

[1]University of Georgia, Alpharetta, GA, United States, [2]Walden University, Minneapolis, MN, United States

In January 2011, symptoms of nausea, abdominal cramps, and diarrhea were reported in a 250-bed skilled nursing facility. Investigations revealed that these symptoms were present in some residents and employees as far back as two days prior, but they were thought to be isolated cases. Upon realization that the cases reported could be an outbreak, immediate actions were put in place to contain the outbreak and to prevent its spread throughout the facility. Immediate actions that were taken included: restrictions of all residents with symptoms in their rooms; staff education that include nursing, environmental, therapists and other caregivers; increasing hand hygiene; restricting visitors to the facility. Sick visitors were asked to stay away; signs were posted on all floors; increasing frequency of cleaning of "high touch" surfaces; using bleach to clean high touch surfaces; notification of the County Department of Health; continued surveillance for immediate identification of new cases and monitoring of old ones; hydration protocol for affected residents to prevent debilitating effects of the virus; sending of samples to the County Department of Health for definitive identification; restrictions of affected employees from care areas; education provided by County Public Health Nurse on the first week of this outbreak. Overall, the outbreak involved over 70 facility residents and the following departmental staff: Nursing, Nutritional Services, Office and Maintenance. On the average, the symptoms lasted about 48 hours for each individual. The norovirus gastroenteritis transmission subsided once the control recommendations (above) were implemented within three weeks after the index case occurred.

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INFLUENZA A VIRUS IN SWINE FARMS FROM GUATEMALA: EVIDENCE OF ZOONOTIC TRANSMISSION FROM HUMANS

Ana S. Gonzalez Reiche[1], Ana L. Ramirez[2], María L. Müller[3], Silvia M. Sosa[3], David Orellana[4], Daniel R. Perez[1]

[1]University of Maryland, College Park, MD, United States, [2]Prince Leopold Institute of Tropical Medicine, Antwerpen, Belgium, [3]Centro de Estudios en Salud, Universidad del Valle de Guatemala, Guatemala, Guatemala, [4]Ministerio de Agricultura Ganadería y Alimentación, Guatemala, Guatemala

In 2009 the emergence of the pandemic H1N1 (pH1N1) strain of influenza A virus (IAV) of potential swine origin, highlighted the need of surveillance of influenza virus in pigs. In Central America, Guatemala is the country with the largest pork production; however the circulation of IAV in the swine population has not been investigated in detail. The main objectives of this study were to determine the presence of IAV in the swine population in Guatemala and identify the circulating subtypes including pH1N1. Two nation-wide multistage random surveys for IAV were conducted. Nasal swabs and blood samples were collected from swine farms and backyard operations during October 2010 and from June to August 2011. Samples were collected from 171 herds in 2010 (n=500) and 136 herds in 2011 (n=499). Herd prevalence for IAV detected by rRT-PCR was 33% for both years. From rRT-PCR positives 4 viruses were isolated, based on their full genome sequences, 3 were fully pH1N1 and one a fully H3N2 seasonal human-like strain. Additionally, antibodies

against IAV were detected by ELISA with herd prevalences of 17.5 and 5.1% for 2010 and 2011 respectively. The H1N1 and H3N2 subtypes from different genetic clusters (swine and human-like) were detected by hemmaglutination inhibition assay. These results suggest that different IAV circulate in the swine population of Guatemala and that human-animal contact may play a role for the introduction of novel strains into the swine population. Global and local methods were used to establish if spatial correlation exists in the IAV positive swine farms from each year. This study is the first in Guatemala analyzing AIV prevalence and its distribution in swine farms from the country.

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DEVELOPMENT OF SYSTEM FOR THE APPLICATION OF ANTIRABIC VACCINES IN UKRAINE

Serhiy Nychyk, Ivan Polupan, Alina Nikitova, Vitalii Nedosekov

IVM, Kiev, Ukraine

In order to develop and implement an effective program for rabies eradication in Ukraine, a collection of samples of pathological rabies-positive materials selected from 17 animal species and humans was founded in 2008 on the basis of regional veterinary laboratories of Ukraine. The collection is regularly updated and now includes 1,340 samples from all regions of Ukraine. We performed a Molecular-genetic study of the collection material using 156 pathological samples/probes from animals having rabies. We performed PCR tests and virus isolation with further sequencing. The study resulted in the determination of two genetic clusters and their clear geographical division in relation to river Dnieper. The genetic clusters' prevalence mapping for the last 5 years showed that cluster II isolates circulate in the regions with maximum rabies spread. Antirabic vaccine efficiency against the two cluster strains circulating in environment was evaluated on the stage of the work. Commercial vaccines obtained with the employment of the rabies virus vaccine strains SAD (Street-Alabama-Dufferin) and Wistar PM/WI were used for the evaluation. The study showed that all the vaccines were 30 % less effective for the cluster II rabies viruses than for the cluster I viruses. The performed research demonstrated genetic and antigen difference between environmental rabies viruses and the strains used for the vaccines production. The obtained results enable one to assume the low efficiency of antirabic vaccines against genetic cluster II rabies viruses as a potential reason for the high rabies prevalence in certain territories. The study results will be employed for the efficiency elevation of antirabic vaccine use in Ukraine on the basis of differential approach depending on vaccine immunogenic activity as well as geographical distribution of rabies virus strains. The performed work also points at the necessity of the development of new regional rabies virus vaccine strains in future.

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SEARCH OF ANTHROPURGIC REASONS FOR RABIES IN UKRAINE

Serhiy Nychyk, Ivan Polupan, Alina Nikitova, Mykola Ivanov, Vitalii Nedosekov

IVM, Kiev, Ukraine

Rabies is an acute viral encephalomyelitis that affects wild and domestic mammals. Worldwide, human death due to rabies makes approximately 55,000 cases annually. Red foxes (Vulpes vulpes) are natural reservoirs of rabies virus in Ukraine. We used the monitoring and mathematical methods for this research. Despite considerable financial expenses on oral immunization of foxes and parenteral immunization of dogs and cats, considerable results have not been achieved in the fight against rabies in Ukraine. It is observed a tendency to increasing of rabies cases in dogs and cats which are the main source of rabies in people. In epidemic section in Ukraine, cats pose the highest hazard, as they are the basic source of infection for humans - 41.3 %, while the rates for dogs and foxes are 24.1 % and 20.7 %, respectively. The rest cases appear from other undefined contacts. When analyzing data, the most important epidemic route for rabies in Ukraine was defined. This could be depicted as follows - fox→cat→human. Over the last decade, nearly 10.5 thousand cases of rabies in cats were detected (average cats morbidity index in Europe is 13.2 %); herewith, 42 % of these cases were registered in Ukraine. The situation analysis based on the reports from veterinarians and veterinary service representatives in urban areas on quantity of fox bites in animals, showed that one of the reasons was the close location of fox inhabitation to urban areas, and their contacts with cats due to the common nutritive base - murine rodents. One more reason was a weak control over the execution of domestic animals' keeping rules, irresponsibility of their owners especially at suburban areas, and small percentage of cats vaccinated against rabies. For instance, 85 % of cats infected with rabies were kept by owners without timely provided vaccination against rabies. With intent to improve the situation with rabies in Ukraine, it is necessary to strengthen control over the rules for keeping domestic animals, increase responsibility of animals' owners for breaking the rules and ensure complete preventive vaccination of cats in endemic zones.

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APPLICATION OF GUANCID TO ENSURE BIOLOGICAL SAFETY DURING WORK WITH AUJESZKY'S DISEASE VIRUS

V. L. Kovalenko, A. V. Pozymniuk

IVM, Kiev, Ukraine

The purpose of a research was to detect the optimal exposure time for the elimination of Aujeszky's disease agent and define Guancid disinfectant safe concentration for animals and humans. The Guancid active component is polyhexamethyleneguanidine hydrochloride. Pig embryo kidney (PEK) and young pig testicle (YPT) cell culture were cultured on well bottoms of 96-well microplate in the form of a monolayer. Different media (RPMI 1640, DMEM, GLA), series 07 bovine blood serum, and phosphate-buffered saline (PBS) were employed for the cell culturing. The "Clone-B" vaccine strain of Aujeszky's disease agent (ADA) was used as the control virus. In order to define the disinfectant antiviral effect, different concentration Guancid solutions in PBS were employed: 0.01; 0.03; 0.05; 0.1; 0.5; 1.0; 2.0; and 3.0 %. After the solution application to the wells, ADA viral suspension with the activity of 107TCD50/cm3 (a dose of virus that causes cytopathic effect in PEK and YPT cells in 24-28 hours without treatment with disinfectant). The virus-disinfectant interaction was performed within 1-60 minutes. Subsequently, the well content was applied onto the surface of cell monolayer to absorb the virus, which was not inactivated by the disinfectant. After 20-minute contact, the microplate was triply washed with PBS and filled with a supporting medium with 2 % of bovine blood serum and incubated. 16 wells with cell cultures were left without disinfectant as the control. Microscopy of wells was conducted twice a day. Results were assessed prior the moment of degenerative changes in the control wells of the microplate. Virus cytopathic effect on the cell cultures was determined at Guancid solution concentrations of up to 0.05 % and at 60 minute exposure. The disinfectant displayed this effect at 2 and 3 % concentrations and at 20 minute exposure. No virus or disinfectant cytopathic effect was detected after 30-minute exposure using Guancid solution concentrations from 0.1 to 1.0 %. It could be concluded that Guancid concentrations from 0.1 to 1.0 % and 30-minutes exposure should be used to ensure biological safety during work with ADA. The disinfectant could be employed for preventive disinfection in veterinary in the presence of animals and humans.

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GEOGRAPHIC DISTRIBUTION OF ZOONOTIC AND VECTOR-BORNE SELECT AGENTS IN KENYA: A SEROLOGIC SURVEY USING A SUBSET OF SERA SAMPLES COLLECTED THROUGH THE KENYA AIDS INDICATOR SURVEY (KAIS)

Leonard Nderitu[1], Lilian Waiboci[2], Rob Breiman[3], Joel Montgomery[2], John Neatherlin[2], Stella Gikunju[1], Cyrus Wachira[1], Kabura Wamburu[1], Lilian Wakhule[1], Mamo Umuro[4], Margret Mbuchi[5], Alfred Muia[6], Charles Magiri[5], Raymond Nyoka[2], Brian Owino[1], Barry Fields[2]

[1]Kenya Medical Research Institute/Centers for Disease Control and Prevention, Nairobi, Kenya, [2]Centers for Disease Control and Prevention, Nairobi, Kenya, [3]Emory Global Health Institute, Atlanta, GA, United States, [4]National HIV Reference Laboratory, Nairobi, Kenya, [5]Kenya Medical Research Institute/U.S. Army Medical Research Unit, Nairobi, Kenya, [6]Kenya Medical Research Institute, Nairobi, Kenya

Zoonoses are diseases and infections which are transmitted naturally between vertebrate animals and man. Vector-borne diseases are infections that are transmitted by the bite of an infected arthropod. Of all human infectious diseases, 61% are zoonotic while 75% of human Emerging Infectious Diseases (EID) are zoonotic. Out of these zoonoses, 33% have a human to human transmission. The dynamics of population growth and lifestyle increases the human-animal-vector encounters and as such exposing humans to zoonotic infections. The incidence and prevalence information of select zoonotic and vector-borne diseases in Kenya is either limited or unknown even though they have been documented to cause outbreaks. An assessment of exposure levels to select zoonotic and vector-borne agents, will determine the most at risk populations to infections as well as outbreaks and therefore target public health interventions. An assessment of co-infection between the select agents will determine what role do co-infections play in prognosis. A total of 15,853 blood samples were collected and analyzed during the 2007 Kenya AIDS Indicator Survey (KAIS). During data collection, serum from participants that consented to storage of their specimens for future testing were separated into several cryovials for processing, including a "storage vial" which was stored in -70°C for future testing. A nationally representative subset of the samples (1091 specimens) was selected and tested for Anthrax, Brucella, Chikungunya, Dengue, Rift Valley Fever, *Rickettsia* and *Leishmania* by IgG Enzyme Linked Immunosorbent Assay (ELISA). Preliminary findings give an indication of geographical hotspots in some regions in Kenya. Of the 1091 serum samples tested, analysis was done by province, residence (rural/urban) and by wealth quintiles. A Dengue, RVF and Richettsial analysis by province shows coast province to have the highest prevalence of antibodies against the 3 select agents followed by North Eastern. The least affected province is Rift Valley. In relation to residence, the rural dwellers were more affected than their urban counterparts. When analyzed by wealth quintiles, the lowest quintile was seen to have the highest prevalence, while the middle quintile had the lowest prevalence of antibodies against the select agents.

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CHARACTERIZATION OF NEUTRALIZING ANTIBODY RESPONSES FOLLOWING NATURAL PRIMARY INAPPARENT AND APPARENT DENGUE VIRUS INFECTIONS

Kizzmekia S. Corbett[1], Hasitha Tissera[2], Dharshan de Silva[3], Aravinda M. de Silva[1]

[1]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [2]Sri Lanka Ministry of Health, Colombo, Sri Lanka, [3]Genetech Research Institute, Colombo, Sri Lanka

Dengue virus is the most significant arthropod-borne virus of humans. Primary dengue infection induces both neutralizing antibodies towards the infecting dengue serotype and cross-reactive non-neutralizing antibodies to other dengue serotypes. The theory of antibody dependent enhancement predicts that cross-reactive antibodies enhance secondary dengue infections, thus resulting in severe disease. During a pediatric fever surveillance cohort study in Colombo, Sri Lanka, sera samples were collected at regular yearly intervals and during and soon after dengue fever episodes. Here, we report on studies that were conducted, using prospectively-collected samples from that cohort, to compare the quality and quantity of dengue-specific antibodies in children with inapparent and apparent infection. Both dengue-specific IgG levels and neutralizing antibody responses induced by primary inapparent and apparent infections were similar. We followed primary dengue cases for up to two years to hone in on the specifics of neutralizing antibody decay over time. Primary infections induced broad-neutralizing antibodies that gradually became monospecific to the infecting serotype over time. The presence of dengue-specific IgM was correlated with broad neutralization. In children exposed to secondary infections, we observed that children with pre-existing monospecific neutralizing antibody responses were more likely to develop fever upon a secondary dengue infection than children with broadly neutralizing antibody pre-existing responses. In all, our findings provide unique insight about development and timing of the neutralizing antibody response following natural primary dengue infection and how such a neutralizing antibody response may influence fever outcome upon secondary infection.

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MUTATIONS THAT MODULATE DENGUE VIRUS "BREATHING" HAVE A SIGNIFICANT IMPACT ON SENSITIVITY TO ANTIBODY-MEDIATED NEUTRALIZATION

Kim A. Dowd[1], Swati Mukherjee[1], Richard Kuhn[2], Michael S. Diamond[3], Daved Fremont[3], Ted Pierson[1]

[1]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [2]Purdue University, West Lafayette, IN, United States, [3]Washington University School of Medicine, St. Louis, MO, United States

Flaviviruses explore multiple conformations via the structural dynamics of viral envelope proteins in the virion. This adds complexity to the antigenic surface of the virion, as virus "breathing" varies the epitopes available for antibody (Ab) binding. A recent study explored the structural basis for genotypic differences in the neutralization potency of a DENV-1 specific mAb (Austin et al., PLOS Pathogens, 2012). mAb E111 binds a poorly exposed domain III epitope on the envelope (E) protein and neutralizes strain 16007 >4000x better than the related strain WP. This result could not be explained by differences in the affinity of E111 for each of these strains. Instead, the ensemble of structures sampled by these two viruses was hypothesized to differ. To further investigate differences in the "breathing" of these two DENV strains, reciprocal WP and 16007 mutants were generated that individually expressed all 13 amino acid differences in the E protein. Using DENV reporter virus particles, these variants were tested for their stability in solution (intrinsic decay) and neutralization sensitivity to a panel of mAbs. Strikingly, differences in the behavior of WP and 16007 mapped to E protein residue 204, located well outside the E111 epitope in domain III. The intrinsic decay rate of WP was ~2x greater than 16007; this difference could be reversed in a reciprocal fashion in the presence of this 204 substitution. The large difference in neutralization sensitivity of these two strains to mAb E111, and a related domain III mAb E98, was significantly modulated by the same residue. Our results demonstrate that neutralization susceptibility can be altered in an epitope-independent manner by subtle mutations (K→R) that alter the overall structural ensemble. That different conformational ensembles of flaviviruses can affect the landscape available for Ab binding, as well as virus stability, has important implications for vaccine development and antibody mapping studies.

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THE ANTIGENIC DETERMINANTS OF SEROTYPE SPECIFICITY FOLLOWING NATURAL DENV-3 AND DENV-4 INFECTION

William B. Messer[1], Ruklanthi de Alwis[2], Scott Royal[3], Boyd Yount[3], Jeremy P. Huynh[1], Ralph S. Baric[3], Aravinda M. de Silva[2]

[1]Oregon Health and Sciences University, Portland, OR, United States, [2]University of North Carolina Chapel Hill School of Medicine, Chapel Hill, NC, United States, [3]University of North Carolina Chapel Hill School of Public Health, Chapel Hill, NC, United States

Dengue virus (DENV) occurs as four serotypes, DENV-1 through DENV-4, and is the most important arthropod-borne viral disease of humans worldwide. Infection with one serotype confers protective immunity to that serotype but not the remaining serotypes-rather, subsequent infection with a heterotypic serotype is associated with an increased risk of severe disease. Despite its worldwide importance, the antigenic determinants on each DENV serotype targeted by protective human antibodies have not been well defined. This knowledge gap has significantly hampering vaccine development. We have recently described the hinge region between domain I and II of the dengue E protein as a target of some human monoclonal antibodies that neutralize DENVs. In the current study we have transplanted the EDI-II hinge between serotypes to determine if this region is the main target of serotype specific neutralizing Abs that develop following primary DENV infections. Transplantation of EDI-II hinge from DENV-4 into DENV-3 leads to a near complete loss of DENV-3/4ic neutralization by monotypic DENV-3 human immune sera and the near complete gain of sensitivity to neutralization by monotypic DENV-4 sera. These results have important implications for vaccine design strategies as well as basic studies of dengue virus biology, immunity, and immunopathogenesis.

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STUDY OF EPITOPES, AVIDITY AND NEUTRALIZING POTENCY OF FLAVIVIRUS GROUP-REACTIVE HUMAN MONOCLONAL ANTIBODIES DERIVED FROM SECONDARY DENGUE VIRUS INFECTION

Wei-Kung Wang[1], Wen-Yang Tsai[1], Chih-Yun Lai[1], Yi-Chieh Wu[1], Hong-En Lin[2], Carolyn Edwards[3], Amonrat Jumnainsong[3], Srisakul Kliks[4], Scott Halstead[4], Juthathip Mongkolsapaya[3], Gavin R Screaton[3]

[1]Tropical Medicine, JABSOM, University of Hawaii at Manoa, Honolulu, HI, United States, [2]Microbiology, National Taiwan University, Taipei, Taiwan, [3]Imperial College of London, London, United Kingdom, [4]Pediatric Dengue Vaccine Initiative, International Vaccine Institute, Seoul, Republic of Korea

The envelope (E) protein of dengue virus (DENV) is the major target of neutralizing antibodies (Abs) and vaccine development. Previous studies of polyclonal human sera after DENV infection revealed that a significant proportion of anti-E Abs were cross-reactive to all four DENV serotypes and to one or more other flaviviruses, known as group-reactive (GR). Studies of mouse anti-E monoclonal antibodies (mAbs) reported that GR mAbs were weakly or non-neutralizing compared with type-specific mAbs; GR response was thus regarded as useless for vaccine strategy. The epitopes of human GR mAbs remain largely unknown. In this study, we investigated the epitopes, binding avidity and neutralization potency of 32 human GR anti-E mAbs. The epitopes involved either fusion loop (FL) residues in E protein domain II only or both FL and bc loop residues in domain II; these residues were highly conserved by different flaviviruses and absolutely conserved by the four DENV serotypes. The neutralization potency and binding avidity of GR mAbs derived from secondary DENV infection were stronger than those derived from primary infection. Analysis of repertoire of anti-E mAbs dereived from patients with primary DENV infection revealed that the majority were GR, low avidity and weakly neutralizing, whereas those from secondary DENV infection were primarily GR, high avidity and potent neutralizing. Our observations suggest the weakly neutralizing GR anti-E Abs generated from primary DENV infection become potent neutralizing against four serotypes after secondary infection. The finding that dengue immune status of host affects the quality of cross-reactive Abs generated may have implications for different strategies of DENV vaccine.

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QUANTIFICATION OF TYPE I INTERFERON SIGNALING IN CELLS INFECTED WITH FIELD STRAINS OF DENGUE VIRUS

Freddy A. Medina[1], Giselle Torres[2], Gilberto A. Santiago[1], Juan F. Medina[1], Luis M. Santiago[1], Amanda J. Chase[3], 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

Dengue virus (DENV), as well as other flaviviruses, circumvent the anti-viral response induced by type I interferon (IFN-α/β) by blocking key players of the JAK/STAT pathway. The relevance of the IFN-α/β system with regards to pathogenic outcomes has been highlighted in gene expression studies of DENV-infected patients showing suppression of interferon stimulated genes in patients with severe dengue. Some studies have suggested that not all DENV or flaviviruses are capable of blocking IFN-α/β signaling. Furthermore, studies of JEV and WNV have suggested a correlation between disease severity and the ability to inhibit IFN-α/β signaling. We have compared the relative inhibition of IFN-α/β signaling by DENVs using a new method that combines flow cytometry and a four-parameter logistic regression model. Clinical isolates from all DENV serotypes and isolates encompassing the five DENV-2 genotypes (Asian, American, Asian/American, Cosmopolitan, and Sylvatic) were selected and analyzed for their IFN-α/β blocking ability. We used the prototypical DENV-2 strain 16681 as a reference strain to normalize the quantitation of IFN-α/β inhibition by DENVs. The inhibitory effect of other DENVs on STAT1 phosphorylation was compared to 16681 using calculations obtained from a four-parameter logistic (4PL) model. All of the DENV serotypes and DENV-2 genotypes analyzed were able to inhibit STAT1 phosphorylation. Modest differences were observed in DENV-3 and DENV-2 sylvatic viruses. We were unable to correlate the relative strength of DENVs to inhibit IFN-α/β signaling with their plaque size or replication capacity. The quantitative method we developed allows us to determine the relative IFN-α/β blocking ability among DENV strains. Contrary to previously published studies of DENV and other flaviviruses, the majority of DENV strains analyzed in this study show a highly conserved ability to inhibit IFN-α/β signaling with a similar magnitude to that observed with DENV strain 16681. Therefore, the probability of correlating pathogenic outcomes in dengue to IFN-α/β signaling inhibition appears to be slim.

1402

EXPLORING THE MECHANISM AND SIGNIFICANCE OF CELL TYPE-DEPENDENT NEUTRALIZATION OF FLAVIVIRUSES

Swati Mukherjee[1], Kimberly A. Dowd[1], Carolyn J. Manhart[1], Anna P. Durbin[2], Stephen S. Whitehead[1], Ted C. Pierson[1]

[1]National Institutes of Health, Bethesda, MD, United States, [2]Johns Hopkins School of Public Health, Baltimore, MD, United States

Flaviviruses assemble at and bud into the endoplasmic reticulum as immature virions containing two glycoproteins, envelope (E) and premembrane protein (prM), arranged in heterotrimeric spikes. Virion maturation involves the cleavage of prM by the cellular serine protease furin. While this cleavage is required for infectivity, it may be inefficient, leading to release of partially mature virions with uncleaved prM. The maturation state of the virion has been shown previously to impact neutralization via changes in epitope accessibility. In this study, we explored the possibility that virion maturation may contribute to cell type-dependent neutralization patterns observed with many monoclonal antibodies (mAbs). We characterized the neutralization activity of a panel of mAbs using multiple target cell types. Several mAbs were significantly

less potent when assayed on Vero or BHK cells, as compared to Raji cells expressing DC-SIGNR; antibody dose-response curves revealed a resistant fraction reminiscent of our studies with antibodies sensitive to the maturation state of the virion. Our data revealed that the apparent inability of antibodies to neutralize WNV when assayed on Vero or BHK was due to the differential impact of uncleaved prM on the specific infectivity of the virus on a given cell type rather than the capacity of the antibody to block infection per se. prM+ viruses are under-represented in neutralization studies using the Vero/BHK cellular substrates typically used in neutralization assays. Analysis of sera from recipients of two live-attenuated dengue virus vaccines revealed a strong correlation between the impact of virion maturation and cell-type dependent patterns of neutralization. The neutralizing potential of cross-reactive responses may be significantly under-represented by the "gold-standard" plaque reduction neutralization test that employs Vero cells.

1403

COHERENT IMMUNE REPERTOIRE SIGNATURES IN HUMAN DENGUE

Poornima Parameswaran[1], Yi Liu[2], Krishna Roskin[3], Katherine Jackson[3], Vaishali P. Dixit[3], Ji-Yeun Lee[3], Karen Artiles[3], Simona Zompi[1], Maria José Vargas[4], Birgitte B. Simen[5], Bozena Hanczaruk[5], Kim R. McGowan[5], Muhammad A. Tariq[6], Nader Pourmand[6], Daphne Koller[2], Angel Balmaseda[4], Scott Boyd[3], Andrew Z. Fire[7], Eva Harris[1]

[1]Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States, [2]Department of Computer Science, Stanford University, Stanford, CA, United States, [3]Department of Pathology, Stanford University, Stanford, CA, United States, [4]Laboratorio Nacional de Virología, Centro Nacional de Diagnóstico y Referencia, Ministerio de Salud, Managua, Nicaragua, [5]454 Life Sciences, A Roche Company, Branford, CT, United States, [6]Baskin School of Engineering, University of California Santa Cruz, Santa Cruz, CA, United States, [7]Departments of Pathology and Genetics, Stanford University, Stanford, CA, United States

Dengue, caused by dengue virus (DENV), is the most prevalent mosquito-transmitted viral disease of humans. The lack of early prognostics, licensed vaccines and therapeutics contributes to tremendous disease burden in endemic areas. In this study, we employed high-throughput sequencing methodologies to capture B cell-associated rearranged immunoglobulin variable heavy chain (V_H) signatures in peripheral blood mononuclear cells (PBMCs) from individuals enrolled in two ongoing dengue studies in Nicaragua. PBMCs were sampled from 44 dengue patients during acute symptomatic dengue (2-5 days post-symptom onset, "dpo"), convalescence (7-47 dpo) and post-convalescence (~180 dpo); 8 individuals with non-dengue febrile illness during the acute phase of disease; and 8 healthy individuals with no prior history of dengue. In addition, an independent set of 16 individuals with symptomatic dengue was sampled during the acute phase of illness. Analysis of V_H sequences from total PBMCs revealed clonal B cell expansion in acute dengue that was greater in secondary than primary DENV infections and not observed in convalescent and post-convalescent samples. We also identified convergent DENV-specific antibody sequences within the hypervariable complementarity determining region 3 (CDR3) that define prevalent and specific indicators of DENV infection; these CDR3 signatures were present in acute symptomatic dengue, significantly reduced after clearance of DENV infection, and not observed in non-dengue samples. The convergent CDR3 regions originated from distinct V_H sequences that were encoded by multiple V genes and were derived from B cell populations that had undergone affinity maturation and accumulated somatic mutations in response to DENV infection. These CDR3 regions and their associated CDR2 and CDR1 sequences have similar amino acid physiochemical profiles that uniquely position them as immune repertoire indicators in human dengue. This is the first report of convergent antibody sequences elicited in response to dengue, and, notably, in response to any natural infection in humans. Similar approaches using samples from individuals infected with different DENV serotypes (and genotypes) could facilitate identification of serotype-specific (and possibly genotype-specific) immune repertoire signatures. Future efforts will also be directed at assessing the antigen specificities of these convergent antibodies.

1404

INCIDENCE OF ACUTE GASTROENTERITIS-ASSOCIATED MORTALITY AMONG CHILDREN UNDER FIVE YEARS OF AGE IN BANGLADESH, 2010-12

Makhdum Ahmed[1], Abdullah Al-Mamun[1], Jaynal Abedin[1], Stephen P. Luby[2], Syed M. Satter[1], Repon C. Paul[1], Marc-Alain Widdowson[2], Katharine Sturm-Ramirez[2], James D. Heffelfinger[2], Mahmudur Rahman[3], Emily S. Gurley[1]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Institute of Epidemiology, Disease Control and Research, Dhaka, Bangladesh

In Bangladesh, diarrhea-related deaths are common among children <5 years. The objective of this study was to estimate acute gastroenteritis-related mortality among children <5 years. We randomly selected 20 unions, the smallest administrative unit in Bangladesh, from the catchment areas of 11 tertiary hospitals from July to December 2012. We used social-networking to identify children aged <5 years who died in the previous two years in the targeted communities. Family members who had taken care of children during the illness preceding death were interviewed about disease symptoms and the types of healthcare sought during the illness. We classified a death as being associated with acute diarrhea if caregivers reported sudden onset of loose, watery stool ≥3 times a day within 14 days of death; we classified a death as related to acute abdomen if caregivers reported sudden onset of abdominal pain without diarrhea within a week of death. We calculated the incidence of acute gastroenteritis-related mortality by dividing the number of deaths associated with acute diarrhea or acute abdomen by the age-specific census population of the study unions. We identified 312 deaths among children <5 years; 41 (13%) following acute diarrhea and 12 (4%) following acute abdomen. Of the 53 children who died with acute gastroenteritis, 43 (81%) were aged <2 years and 26 (49%) were male. The annual incidence of acute gastroenteritis-related deaths per 10,000 children <5 years was 3.7 (95% CI 2.7-4.8), and 3.0 (95% CI 2.2-4.0) for acute diarrhea-related death. Twenty eight of 53 (53%) children died in November to February during 2010-12. Thirty five of 53 (66%) received treatment from certified physicians or hospitals within four days of illness onset and 28 of 53 (53%) died at home. The burden of acute gastroenteritis-associated mortality was highest among children <2 years. The months in which deaths peaked correspond with seasonal peaks of rotavirus circulation in Bangladesh, suggesting that this pathogen may contribute importantly to child deaths. The planned introduction of rotavirus vaccine could substantially reduce childhood mortality in Bangladesh. Many children did not seek care from trained providers, and more than half died even after seeking qualified care, suggesting that quality care may not have been accessible to these children. Improving access to prompt management of childhood gastroenteritis could save lives.

1405

HOME-BASED DIARRHEA CASE MANAGEMENT AND THE RISK OF ALL-CAUSE MORTALITY IN THE KENYA GLOBAL ENTERICS MULTICENTER STUDY (GEMS) SITE

Arthi Vasantharoopan[1], Ciara O'Reilly[2], Richard Omore[3], Karen Kotloff[4], Tamer Farag[4], Myron M. Levine[4], Kayla Laserson[5], Robert F. Breiman[5], Eric D. Mintz[2], Pavani K. Ram[1]

[1]University at Buffalo, Buffalo, NY, United States, [2]Division of Foodborne, Waterborne and Environmental Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Kenya Medical Research Institute/Centers for Disease Control and Prevention, Kisumu, Kenya, [4]University of Maryland, Baltimore, MD, United States, [5]Kenya Medical Research Institute/Centers for Disease Control and Prevention, Kisumu, Kenya and Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, United States

Modeling studies suggest that oral rehydration solution (ORS) may prevent most diarrheal deaths if universal coverage is achieved. Globally, only one-third of children with diarrhea receive ORS, the form of rehydration for diarrhea recommended in the Integrated Management of Childhood Illness guidelines. Achieving 100% ORS use will require substantial financial input and behavior change efforts. To strengthen the case for promoting appropriate diarrhea case management at home, we evaluated whether home-based diarrhea treatment methods reduced the risk of death in children with moderate to severe diarrhea (MSD). At the GEMS Kenya field site, we enrolled children <59 months old with MSD. Health workers asked caretakers about diarrhea case management at home during the time preceding enrollment. Survival status was determined at follow-up 50-90 days after enrollment. We calculated risk ratios (RR) and 95% confidence intervals (CI) to describe associations between the following and death: oral rehydration therapy (ORT) consisting of ORS, recommended home-fluids, or increasing fluids; ORS with or without other fluids; and continued feeding (CF). During 2008-11, 1,476 MSD cases were enrolled; 1,419 (96%) were followed up. At home, 65% of children received one or more forms of ORT, 14% received ORS with or without other fluids, and 19% were offered continued feeding. Between enrollment and follow-up, 52 deaths occurred (case fatality rate 3.7%), 35% of which reportedly occurred < 7 days after enrollment. Children with MSD who later died were more likely to present with slow return skin pinch and restless/irritable mental status at enrollment, and to require intravenous hydration and hospitalization during treatment than those who survived (P<.0001 for all). Home diarrhea case management strategies were not significantly associated with mortality (ORT: RR=0.92 [CI 0.53-1.61], ORS: RR=1.65 [CI 0.87-3.16], and CF: RR=0.45 [CI 0.18-1.12]. In Kenya, children with MSD experienced a high case fatality rate, but few were offered ORS and continued feeding at home. Clinical signs suggesting dehydration were associated with mortality. A small number of deaths and low rates of ORS use and CF reduced our ability to identify protective effects of ORT, ORS, and CF among children with moderate-to-severe diarrhea.

1406

ASSOCIATION BETWEEN ENTEROPATHOGENS, DIARRHEA AND GROWTH IN THE MAL-ED COHORT

James Platts-Mills[1], Benjamin J. McCormick[2], Monica McGrath[2], Mark Miller[2], Jean Gratz[1], Margaret Kosek[3], Alexandre Havt[4], Samie Amidou[5], Anita Zaidi[6], Gagandeep Kang[7], Rashidul Haque[8], Ladaporn Bodhidatta[9], Eric Houpt[1], On behalf of the MAL-ED Network

[1]University of Virginia, Charlottesville, VA, United States, [2]Fogarty International Center, National Institutes of Health, Bethesda, MD, United States, [3]Asociación Benéfica PRISMA, Iquitos, Peru, [4]Federal University of Ceara, Fortaleza, Brazil, [5]University of Venda, Thohoyandou, South Africa, [6]Aga Khan University, Karachi, Pakistan, [7]Christian Medical College, Vellore, Tamil Nadu, India, [8]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [9]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

The pathogenicity of enteric infections is typically defined by their association with diarrhea. However, enteric infection has also been linked to stunted growth and decreased cognitive development. It is not clear whether the pathogens most clearly associated with diarrhea are equally important for these long-term outcomes. We performed an interim analysis of the association between enteropathogens and both diarrhea and poor linear growth in the multisite MAL-ED cohort study. To estimate pathogen-specific burdens of diarrhea, we calculated the population attributable fraction (AF) of diarrhea for each pathogen that had a statistically significant association with diarrhea. We constructed models using nine-month linear growth intervals to estimate the association between enteropathogen infections and linear growth. We then developed pathogen-specific models to determine the relative effect of symptomatic and asymptomatic infections on growth. In the first year of life, the top three causes of community diarrhea were rotavirus (aggregated AF 4%), astrovirus (3%), and enterotoxigenic *E. coli* (ETEC; 3%). *Campylobacter sp.* had the highest burden of diarrhea at three sites (Brazil, Peru, and South Africa) but was not significantly associated with diarrhea at the other sites. In the second year of life, rotavirus (aggregated AF 7%), *Shigella* (4%), astrovirus (4%), ETEC (4%), and *Cryptosporidium* (4%) had the highest burdens of diarrhea. The pathogens associated with poor linear growth were *Campylobacter* (aggregated average height loss of 0.06cm per nine-month interval), *Giardia* (0.03cm) and *Cryptosporidium* (0.01cm). In the pathogen-specific models, most of this growth burden was mediated by asymptomatic infection. Our preliminary findings suggest that the pathogens associated with growth shortfalls are different than those associated with diarrhea. These findings have substantial implications for prioritizing interventions designed to address both the mortality and morbidity associated with these infections in children in low-income countries.

1407

ENTEROAGGREGATIVE *ESCHERICHIA COLI* ASSOCIATED WITH MALNOURISHED CHILDREN IN THE MAL-ED CASE-CONTROL STUDY IN FORTALEZA, CEARÁ, BRAZIL

Aldo A. Lima[1], Ila F. Lima[1], Nadia Boisen[2], Álvaro M. Leite[1], Alessandra F. Moura[1], Noélia L. Lima[1], Alberto M. Soares[1], José Q. Filho[1], Alexandre Havt[1], Francisco S. Junior[1], Reinaldo B. Oriá[1], James P. Nataro[3], Richard L. Guerrant[3]

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

Enteroaggregative *Escherichia coli* (EAEC) is an important enteric pathogen worldwide, but the disease pathophysiology remains obscure. This case-control study aimed to describe the prevalence of EAEC and potential associations of its VRGs with risk or protection to malnourished (moderate to severely underweight children, determined by weight for age Z score (WAZ) <-2) and nourished (age, sex, and neighborhood matched controls

presenting WAZ >-1) Brazilian children. Stool samples were collected from 259 children, 132 cases and 127 controls, aged 6 to 24 months that visited the specialized clinic in infant malnutrition (IPREDE) in Fortaleza, Ceará, Brazil, from Aug/2010 to Jul/2012. The specimens were cultured for *E. coli*, which was screened using microbiological standard methods. *E. coli* strains were tested for EAEC by polymerase chain reaction (PCR). For each sample, a pool of up to 5 single colonies from original MacConkey plates were examined for the EAEC diagnostic genes (aaiC and aatA). Some positive strains were individually analyzed by multiplex PCR to identify 18 VRGs. EAEC (aaiC+ and aatA+) was significantly found in 34% (45/132) of cases and 21% (27/127) of controls (P=0.021). Among these positive strains, 62 EAEC isolates obtained from 19 children, 15 cases and 4 controls, were further investigated by multiplex PCR. All EAEC strains carried at least three of the 18 assayed VRGs. The transcriptional activator aggR was the most common (98.54%), followed by genes encoding the mucinase pic (95.2%) and the hypothetical cryptic protein orf3 (93.5%). Heat-stable toxin EAST-1 and hypothetical hemolysin orf61 genes were strongly associated with cases among the EAEC strains tested (P=0.0003, OR=31.47, 95%CI=1.77-557.8; and P=0.006, OR=8.25, 95%CI=1.89-35.98, respectively). In addition, genes encoding the toxin sat and protease sepA were significantly more detected in controls compared to malnourished children (P=0.0001, OR=0.03, 95%CI=0.002-0.48; and P=0.023, OR=0.21, 95%CI=0.06-0.73, respectively). These data confirm a high prevalence of EAEC strains in the studied population and the higher association with malnourished children. Our plans include completing the EAEC VRGs characterization in all isolates to determine the importance of a combination of these VRGs potential associated with its pathogenesis.

1408

SOCIO-ECONOMIC, MOTHER CHARACTERISTICS AND CHILD CARE RISK FACTORS ASSOCIATED WITH MALNOURISHED CHILDREN IN THE MAL-ED CASE-CONTROL STUDY IN FORTALEZA, CEARÁ, BRAZIL

Aldo A. Lima[1], Álvaro M. Leite[1], Alessandra F. Moura[1], Noélia L. Lima[1], Reinaldo B. Oriá[1], Alberto M. Soares[1], José Q. Filho[1], Alexandre Havt[1], Ila F. Lima[1], Josiane S. Quetz[1], Francisco S. Junior[1], Cláudia B. Abreu[1], Francisco S. Mota[1], Richard L. Guerrant[2]

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

In Fortaleza, located in the poorest region of Brazil, there is a large socioeconomic and cultural disparity that maybe influences the prevalence of malnutrition and its serious consequences for growth and cognitive development in children. The aim of this study was to evaluate the risk factors related to the child and the mother, as well as environmental and socioeconomic factors associated with the development of malnutrition in children in this population. A total of 345 children aged 6 to 24 months that visited the specialized clinic in infant malnutrition (IPREDE) in Fortaleza, Ceará, Brazil, from Aug/2010 to Mar/2013 were enrolled in this study. Cases (N=165) were defined as children with weight-for-age (WAZ) z-score less than -2 and control (N = 180) healthy children with WAZ more than -1. The children were monitored for anthropometric parameters and morbidity at baseline and at quarterly visits for a year. Specific questionnaires were developed and used for data acquisition on risk factors. The children in both groups did not differ by gender and age. The controls had a better birth weight, length and head circumference compared to cases (p<0.01). In relation to breastfeeding the controls showed a significant increase in the percentage in the first 24 hours (p<0.01) as well use more colostrum (OR=0.24; CI95%:0.11-0.55; p<0.01) than the cases and maintained breastfeeding longer than cases (OR=0.60; CI95%:0.38-0.94; p=0.01). The pattern of introducing liquids in the first three months of life was significantly more favorable for the controls (p<0.01). Regarding to mother´s educational level there was a significant increase in school years in controls compared with cases (OR=0.38; CI95%:0.17-0.87; p=0.01). The controls had a higher percentage in the use of piped water in the house compared to the cases (OR=2.16; CI95%:0.93-5.12; p=0.05). A multivariate hierarchical analysis is needed to determine the influence of variables on the combined outcomes and it is in progress now. In conclusion, the data showed that the weight, length and head circumference at birth, delayed initiation of breastfeeding after birth, deficit in the colostrum intake, low maternal education and poor quality of sanitation are risk factors associated with infant malnutrition in this population.

1409

CATCH-UP GROWTH OCCURS WHEN DIARRHEA BURDEN IS LOW IN EARLY CHILDHOOD

Stephanie A. Richard[1], Robert E. Black[1], Robert H. Gilman[1], Richard L. Guerrant[2], Gagandeep Kang[3], Claudio F. Lanata[4], Kåre Mølbak[5], Zeba A. Rasmussen[6], R. Bradley Sack[1], Palle Valentiner-Branth[5], William Checkley[7]

[1]Johns Hopkins University, Bloomberg School of Public Health, Baltimore, MD, United States, [2]University of Virginia School of Medicine, Charlottesville, VA, United States, [3]Christian Medical College, Vellore, India, [4]Instituto de Investigacion Nutricional, Lima, Peru, [5]Statens Serum Institut, Copenhagen, Denmark, [6]Fogarty International Center, National Institutes of Health, Bethesda, MD, United States, [7]Johns Hopkins University, School of Medicine, Baltimore, MD, United States

Diarrhea and linear growth faltering continue to burden low-income countries and are among the most important causes of illness and death during early childhood. Diarrhea is thought to adversely affect linear growth, but catch-up growth can occur if no further insults are experienced. We sought to characterize catch-up growth in relation to frequency of diarrhea in a multi-site setting. Using longitudinal anthropometry and diarrheal surveillance from seven cohort studies in four countries, we examined the relationship between diarrhea prevalence and length v*eloci*ty in 3- to 6-month periods using linear mixed effect models. The v*eloci*ty during each period was calculated from the models as a function of age using linear splines. We incorporated the longitudinal prevalence of diarrhea in both current and previous periods into the model. Diarrhea during the current period was associated with slower growth in all age groups except for 0-3 months. Faster (catch-up) growth in length was observed in children with no diarrhea in the current period following a period in which diarrhea was experienced (6.01-12 month age group: 0.03 mm per month for each percent diarrhea prevalence in the previous period (95% CI: 0.009, 0.05); 12.01-18: 0.03 (0.02, 0.05); 18.01-24: 0.03 (0.002, 0.06)). Similar results were observed when weight was the outcome variable. When diarrhea episodes are followed by diarrhea-free periods in the first two years of life, catch-up growth is observed. Catch-up growth can allow children to regain their original trajectories given no or reduced diarrhea burden in subsequent periods. Diarrhea burdens are high throughout the first two years of life in developing countries, therefore reducing the likelihood of catch-up growth. Extending diarrhea-free periods may result in improved catch-up growth and a lower level of stunting. Diarrhea-free periods can be attained through expanded implementation of well-documented interventions (e.g., rotavirus vaccine, breastfeeding, zinc supplementation, and improved water and sanitation).

1410

PREVALENCE OF NON-*JEJUNI/COLI CAMPYLOBACTER* SPECIES DETECTED BY ENZYME IMMUNOASSAY AND CULTURE IN THE MAL-ED COHORT STUDY

James A. Platts-Mills[1], Jie Liu[1], Jean Gratz[1], Esto Mduma[2], Caroline Amour[3], Ndealilia Swai[3], Mami Taniuchi[1], Sharmin Begum[4], Julian Flores[5], Ben Parker[6], James Fox[7], Margaret Kosek[8], Rashidul Haque[4], Eric Houpt[1]

[1]University of Virginia, Charlottesville, VA, United States, [2]Haydom Lutheran Hospital, Haydom, United Republic of Tanzania, [3]Kilimanjaro Clinical Research Institute, Moshi, United Republic of Tanzania, [4]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [5]Asociación Benéfica PRISMA, Iquitos, Peru, [6]Johns Hopkins University, Baltimore, MD, United States, [7]Massachusetts Institute of Technology, Boston, MA, United States, [8]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Methods for detection of *Campylobacter* species include culture, enzyme immunoassay (EIA) and PCR. A large discrepancy in detection by culture and EIA was noted in diarrheal and asymptomatic surveillance samples tested by both methods in the MAL-ED cohort study: 4.0% vs. 32.4% respectively in Bangladesh and 4.4% vs. 20.0% in Peru. To better understand this discrepancy, we randomly selected a total of 436 samples comprised of diarrheal cases and matched controls from children 0-12 months of age from Tanzania, Bangladesh, and Peru. According to the study protocol, all samples had previously been tested by the ProSpecT *Campylobacter* ELISA as well as by selective culture in Bangladesh and Peru. Additionally, we tested all samples with a duplex PCR assay for *C. jejuni/coli* (cadF) and *C.* species (16S rRNA). 71.6% of EIA positive samples were positive for cadF and 100% were positive for *Campylobacter* 16S rRNA, suggesting that EIA positivity was associated with non-jeuni/*coli Campylobacter* species. Next, we used 16S rRNA-based primers to sequence 60 EIA-positive samples for which the 16S rRNA quantification cycle (Cq) was at least 10 cycles lower than the cadF Cq. A sequence was successfully obtained for 50 of the samples, which most closely matched known 16S rRNA from *C. hyointestinalis* subsp. *lawsonni* (48%), *C. troglodytis* (30%), *C. jejuni/coli* (16%), and *C. upsaliensis* (6%). Of these, 7 were positive by selective culture, of which 4 were *C. hyointestinalis* subs *lawsonni*, 2 were *C. troglodytis*, and one *C. jejuni/coli. C. hyointestinalis* was the most frequently matched species in Tanzania and Peru, and *C. troglodytis* was the most frequently matched species in Bangladesh. Eight 16S positive/cadF positive samples with a less than 10x discrepancy in 16S and cadF burden were also sequenced, which most closely matched *C. jejuni/coli* (87.5%) and *C. troglodytis* (12.5%). PCR reveals a high burden of non-*jejuni/coli Campylobacter* in infants in these settings, some of which is detected by enzyme immunoassay and culture. *C. hyointestinalis* subsp. *lawsonni* is of porcine origin while the reservoir for *C. troglodytis* is not clearly established. We would estimate that approximately 10-20% of EIA positive samples from this age group from these sites represent non-*jejuni/coli Campylobacter* species, the clinical importance of which is not known.

1411

COMPARISON BETWEEN POST-TREATMENT REACTIONS AFTER DEC OR IVERMECTIN IN SUBJECTS WITH LOIASIS

Jesica A. Herrick[1], Fanny Legrand[1], Raceline Gounoue[2], Jean Bopda[3], Steve Bickmen Tchana[3], Bienvenu Etogo Ondigui[2], Céline Montavon[4], Thomas Nutman[1], Joseph Kamgno[3], Amy D. Klion[1]

[1]Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [2]Université de Yaounde, Yaounde, Cameroon, [3]Center for Research on Filariasis and Other Tropical Diseases, Yaounde, Cameroon, [4]Institut de recherche pour le développement; Faculté des Sciences, Montpelier, France

Diethylcarbamazine (DEC) treatment of loiasis is complicated by severe adverse reactions that are related to the number of circulating microfilariae (MF). The cause of these reactions in unknown, but they are accompanied by a dramatic increase in IL-5 and absolute eosinophil count (AEC). Clinically similar reactions have been seen following mass drug administration of ivermectin (IVM) for control of onchocerciasis in *Loa*-endemic areas and impact the success of filariasis control programs. To directly compare post-treatment responses following DEC and IVM, we randomized 12 subjects with loiasis and <2000 MF/mL blood to receive single-dose DEC (8 mg/kg) or IVM (200 mcg/kg). Adverse events (AE), AEC and MF counts were assessed at baseline, 4, 8 and 24 hours, and 2, 3, 5, 7, 9, and 14 days. Serum was stored at all time points for additional analyses. Baseline characteristics were comparable between the two treatment groups. All study subjects experienced mild to moderately severe AEs in the first 3 days post-treatment that were similar in character and frequency in the two groups. In the DEC group, AEC decreased from baseline levels within 24 hours in all subjects (from GM 3269/μL to 1139/μL, p=.03). This was followed by a slow rise in AEC, peaking between days 2 and 9. In contrast, all subjects in the IVM group experienced a transient increase in AEC during the first 24 hours (GM 1761/μL to 4081/μL, p=.03) with return to baseline levels by day 2. MF counts decreased dramatically in all subjects by 24 hours post-therapy (GM 1074 to 0 MF/ml in the DEC group, p =.03, and 355 to 32 MF/ml in the IVM group, p=.03), although the proportion of subjects with measurable MF counts was greater in the IVM group at all time points (p<0.05 at days 1, 3 and 5). These data suggest that DEC and IVM have differing effects on microfilarial clearance and post-treatment eosinophilia. This may have important implications with respect to interventions to prevent post-treatment reactions.

1412

COMPARISON OF THE IMMUNE RESPONSE PROFILE IN SUBJECTS WITH *LOA LOA* INFECTION AFTER A SINGLE-DOSE OF DIETHYLCARBAMAZINE OR IVERMECTIN

Fanny Legrand[1], Jesica Herrick[1], Celine Montavon[2], Michelle Makiya[1], Raceline Gounoue[3], Jean Bopda[4], Steve Bickmen Tchana[4], Simon Metenou[1], Godwin Nchinda[5], Joseph Kamgno[4], Amy D. Klion[1]

[1]Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [2]Institut de recherche pour le développement, Montpellier, France, [3]Faculté des Sciences, Université de Yaounde, Yaounde, Cameroon, [4]Center for Research on Filariasis and Other Tropical Diseases, Yaounde, Cameroon, [5]Laboratory of Immunology, The Chantal Biya International Reference Centre for Research on HIV/AIDS Prevention and Management (CIRCB), Yaounde, Cameroon

Post-treatment reactions can occur in patients with loiasis following administration of either diethylcarbamazine (DEC) or ivermectin (IVM) and are believed to be due to host immune responses to dying microfilariae (MF). Although a dramatic increase in IL-5 driven eosinophilia has been described post-DEC treatment of loiasis, little is known about immune responses post-IVM. To compare the immune responses following administration of these two drugs, 12 subjects with loiasis and ≤2000 MF/mL blood were randomized to receive a single oral dose of IVM (200 mcg/kg) or DEC (8 mg/kg). Complete blood counts were performed and serum collected for mediator analysis at baseline, 4, 8 and 24 hours, 2, 3, 7 and 9 days post-treatment. Whole blood flow cytometry was performed at baseline, 1 and 3 days post-treatment to assess T cell and eosinophil activation. In the DEC group, the absolute eosinophil count (AEC) decreased from baseline levels at 8 hours in all subjects; whereas, all subjects in the IVM group experienced a transient increase in AEC during the same time frame. The absolute neutrophil count increased at 8 hours post-treatment in all subjects, regardless of treatment group. Eosinophil surface expression of CD69 increased in 11/12 subjects on day 1 post-treatment. In contrast, eosinophil surface expression of CD25 increased by a median of 96% in the subjects who received DEC, but decreased by a median of 60% in the subjects who received IVM. Similar discordance was seen with respect to CD25 expression on CD4+ T cells, with a 19% increase in the DEC group and a 46% decrease in the IVM group. Serum

IL-5 levels rose significantly post-treatment in all subjects, but peaked earlier in subjects who received DEC compared to those who received IVM (8 hours vs. 2 days). Serum IL-10 and MCP-1 levels increased post-treatment only in the DEC group. The observed differences in immunologic profiles of subjects with loiasis who received DEC as compared to those who received IVM suggest that these two drugs may exert their microfilaricidal effects through different mechanisms.

1413

IS *ONCHOCERCA VOLVULUS* SUBOPTIMAL RESPONSE TO IVERMECTIN A RESULT OF SELECTION UNDER IVERMECTIN PRESSURE? INSIGHTS FROM A STUDY COMPARING IVERMECTIN AND MOXIDECTIN IN AREAS WITHOUT PRIOR IVERMECTIN MASS TREATMENT

Didier Bakajika[1], Eric Kanza[2], Hayford Howard[3], Nicholas Opoku[4], Jean-Pierre L. Tchatchu[1], Kambale Kataliko[2], Mawolo Kwapor[3], Simon K. Attah[4], Michel Vaillant[5], **Piero L. Olliaro**[6], Christine M. Halleux[6], Annette C. Kuesel[6]

[1]Centre de Recherche en Maladies Tropicales de l'Ituri, C.R.M.T- ITURI, Hôpital Général de Référence de Rethy, Rethy, Democratic Republic of the Congo, [2]Centre de Recherche Clinique de Butembo (CRCB), Université Catholique du Graben (UCG), Butembo, Democratic Republic of the Congo, [3]The Liberian Institute for Biomedical Research, Clinical Research Center Bolahun, Bolahun, Liberia, [4]Onchocerciasis Chemotherapy Research Centre, Hohoe, Ghana, [5]CCMS CRP-SANTE, Luxembourg, Luxembourg, [6]UNICEF/UNDP/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases, Geneva, Switzerland

Control and progress towards elimination of onchocerciasis in Africa currently rely on annual ivermectin (IVM) mass treatment (CDTI). Concern has been raised about longterm CDTI selecting for parasites with 'suboptimal response', i.e. higher skin microfilaria (mf) levels than considered 'adequate response', threatening control objectives. We analysed data from a study in Ghana, Liberia and DRC in areas without CDTI for indication of 'suboptimal' response. IVM and moxidectin (moxi) had been given to 494 and 978, respectively, males and females ≥12 years with ≥10 mf/mg skin. For the 97.2% of IVM treated and the 96.6% of moxi treated with 12 months follow up, baseline levels were 41.1±31 and 39.1±30.9, respectively (mean±SD mf/mg). Ivermectin treated: 1, 6, and 12 months post dose, mf levels were >17% of baseline in 10.2%, 14.1% and 46%, and >40% of baseline in 4.6%, 3.5% and 18.3% of subjects. Maximum levels were 150%, 159% and 375% of baseline, respectively. The % of IVM treated with undetectable levels was 42.7%, 11.3% and 5.2% at 1, 6 and 12 months. Moxidectin treated: 1, 6, and 12 months post-dose, mf levels were >17% of baseline in 0%, 0% and 4.8% and >40% of baseline in 0%, 0%, and 1.1% of subjects. Maximum levels were 8.1%, 8.5% and 58.5 % of baseline, respectively. The % of moxi treated with undetectable levels was 83.2%, 91.6% and 46.5% at 1, 6 and 12 months. The data did not indicate site or pre-dose level dependency. While the higher efficacy of moxi relative to IVM ($p<0.0001$ for all endpoints tested) shows that IVM efficacy is not optimal, comparison of the data of IVM treated with criteria and analyses in the literature shows that significant percentages of 'suboptimal responders' to ivermectin are present in populations which have not been under IVM selection pressure. This suggests that the natural variability of response to IVM is larger than commonly assumed, which needs to be taken into account during the design and analysis of studies on the origin, frequency and potential impact of 'suboptimal' IVM responders.

1414

THE DEVELOPMENT OF THE LYMPHATIC FILARIASIS QUALITY OF LIFE TOOL BANGLADESH (LF-QOL BANGLADESH)

Lynne Zeldenryk[1], Marion Gray[2], Susan Gordon[1], Richard Speare[1], Wayne Melrose[1]

[1]James Cook University, Douglas, QLD, Australia, [2]Southern Cross University, Sunshine Coast, QLD, Australia

Lymphatic Filariasis (LF) is the world's leading cause of physical disability. Despite this, little is known about LF-disability across the stages of disease progression/manifestation, gender, age and socio-economic groups. A lack of quality data on LF-disability impact makes it difficult to develop evidence based interventions targeted to key areas of need and disease stages. A review of tools currently used in the field found that they demonstrably fail to measure the majority of known impacts of LF-disability and are culturally and linguistically inappropriate for LF-endemic populations. A Lymphatic Filariasis Quality of Life Tool was developed through a multi-staged mixed methods research process including a review of known impacts of LF-disability, in-country focus groups, cross-cultural testing and refinement and reliability studies. The final tool, the LF-QOL Bangladesh, is a 72 item, four point response format tool which measures LF-disability experience across four domains: daily activity and participation, body functions, environmental factors (community supports and barriers) and psychological impacts. In-depth in-country cognitive interviewing refined and confirmed the cultural and linguistic validity of the tool. Reliability studies found the overall internal consistency (0.917) and Corrected Item-Total correlation scores (0.91-0.926) to be excellent. The results have implications for disability measurement broadly across neglected tropical diseases (NTDs) and for the development of disability measures for intervention planning and outcome measurement.

1415

CAPACITY STRENGTHENING FOR LYMPHATIC FILARIASIS MORBIDLY MANAGEMENT: EXPERIENCE FROM PANGANI HYDROCECTOMY CAMP

Mwelecele N. Malecela[1], Upendo J. Mwingira[1], Mabula Mchembe[2], Serigne M. Gueye[3], Sunny Mante[4], Wilfred Lazarus[1], Bernard Kilembe[1], Mbutolwe E. Mwakitalu[1], William J. Kisoka[1], Prince Mutalemwa[1], Yusuf Makange[5], Rehema Majjid[6], Charles Mackenzie[7], Sidney Yongolo[2]

[1]National Institute for Medical Research, Dar-es-salaam, United Republic of Tanzania, [2]Muhimbili University of Health and Allied Sciences, Dar-es-salaam, United Republic of Tanzania, [3]Cheik Anta Diop University, Dakar, Senegal, [4]Military Hospital, Accra, Ghana, [5]Pangani District Hospital, Pangani, United Republic of Tanzania, [6]Tanga Regional Hospital, Tanga, United Republic of Tanzania, [7]Michigan State University, East Lansing, MI, United States

The Pangani hydrocelectomy Camp was the first ever organised by the National Lymphatic Filariasis Programme in Tanzania in consultation with Department of surgery Muhimbili University of Health and Allied Sciences (MUHAS) and West African hydrocectomy programme. The Funding for this Camp was through The President Kikwete LF Fund. Village Health Workers (VHW) registered over 400 Patients during MDA, which was followed up by screening by the surgeons at Pangani District hospital and final confirmation from specialist surgeons from MUHAS and PAUSA. As well as the standard clinical examination patients were also examined for presence of microfilaria and Circulating Filarial Antigen (CFA). The camp was organised such that specialist surgeons from tertiary hospitals trained District surgeons who then worked together to carry out the surgeries. A manual from the West African hydrocelectomy Programme was reviewed and adapted for use during this camp. The focus was in the use of the Excision technique, which required the excision of the tunica *vaginalis* to ensure no recurrence. A total of 202 patients with an age range was between 9 and 86 years, were operated on in the 10 days. Out of the 200 patients 101(50.0%) had bilateral 20(10.0%)had hydroceles with hernia

and 5(2.5%)had hydrocele with testicular complications like atrophy, tumour and necrosis. Hydrocele fluid was collected for biochemical analysis and tissue sections of the Tunica *vaginalis* were preserved in formalin for histopathology. The Surgeons trained village health workers who looked after the patients post surgery in the village. A special algorithm on wound care management and danger signs was provided to all VHW and findings recorded in daily diaries, which were reviewed after 7 days. The experience indicated that large-scale camps are useful especially when they involve local personnel at district level. It also showed that Village Health workers could support postoperative care and hence the involvement of Village Health Workers in morbidity management is crucial. The camp was also a great advocacy activity inspiring more men to register for hydrocelectomies.

1416

COMPLIANCE TO LYMPHEDEMA MANAGEMENT TECHNIQUES AND ITS IMPACT ON THE RATE OF ADENOLYMPHANGITIS (ADLA) EPISODES IN KHURDA DISTRICT, ORISSA STATE, INDIA

Katherine E. Mues[1], Michael Deming[2], Aishya Prakash[3], Jonathan Rout[3], LeAnne Fox[2]

[1]Emory University/Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Church's Auxiliary for Social Action, Bhubaneswar, India

Lymphedema management programs have been shown to decrease episodes of ADLA, but the impact of compliance with specific lymphedema management techniques has not been explored in detail. Our objectives were to determine the rate of ADLA episodes over time for patients enrolled in a community-based lymphedema management program and determine predictors of compliance to the program. A community-based lymphedema management program was implemented in Orissa State, India from 2007-2010 by the Indian non-governmental organization, Church's Auxiliary for Social Action, in consultation with the Centers for Disease Control and Prevention. Patients (n=374) were followed over 24 months. The 30-day rate of ADLA episodes decreased from 0.34 episodes per person at baseline to 0.23 episodes per person at 24 months (P=0.0043). From baseline until 24 months after the program began, the average of compliance with each separate lymphedema management technique (limb washing with soap, anti-fungal cream use, elevation of the limb, limb exercise, and use of footwear outdoors) increased from 19.3% (2.9%-41.8%) to 65.4% (34.2%-92.2%) (p<0.0001). Ordinal logistic regression models found increasing age (OR=1.02 [1.01, 1.03]), paid work (OR=1.71 [1.21, 2.41]), and use of a mosquito net (OR=1.45 [1.08, 1.95]) to be significantly associated with compliance to limb washing with soap. Increasing lymphedema stage (OR=1.16 [1.02, 1.30]), increasing age (OR=1.05 {1.04, 1.07]) and increasing number of ADLA episodes in the last 6 months (OR=1.15 [1.06, 1.25]) were associated with compliance to wearing footwear outside the home. This study demonstrates improvement in ADLA episodes within a community-based lymphedema program. In addition, it illustrates characteristics of persons who complied with lymphedema management techniques and can assist programs in targeting those who may be less compliant in lymphedema management programs.

1417

REPURPOSED LABORATORY EQUIPMENT PROVIDE A FIELD-FRIENDLY, POINT-OF-CARE METHOD FOR QUANTIFYING *LOA LOA* MICROFILARAEMIA IN ADVANCE OF "TEST AND (NOT) TREAT" STRATEGY PREVENTION OF POST-TREATMENT SERIOUS ADVERSE EVENTS

Sasisekhar Bennuru[1], Sebastien D. Pion[2], Joseph Kamgno[3], Samuel Wanji[4], Thomas B. Nutman[1]

[1]National Institutes of Health, Bethesda, MD, United States, [2]Filariasis and Other Tropical Diseases Research Center, Yaounde, Cameroon and UMI 233, Institut de Recherche pour le Développement (IRD), Montpellier, France, [3]Filariasis and Other Tropical Diseases Research Center, Yaoundé, Cameroon and Faculty of Medicine and Biomedical Sciences, University of Yaoundé, Yaoundé, Cameroon, [4]Research Foundation in Tropical Diseases and the Environment and Department of Microbiology and Parasitology, University of Buea, Buea, Cameroon

Administration of ivermectin as part of mass drug administration (MDA) campaigns for onchocerciasis and/or lymphatic filariasis in areas co-endemic for *Loa loa* has resulted in severe post-treatment adverse events (SAEs) including encephalopathy and death. This has led to the suspension of MDA in some of these co-endemic areas of Central Africa. One simple, potential solution aimed at preventing *Loa*-associated post-treatment SAEs is to identify and exclude individuals at risk (high levels of microfilaraemia) from the MDA in a program termed 'Test and (Not) Treat" (TNT). We describe the adaptation and optimization of an existing technology for a rapid, point-of-care method for quantifying microfilariae in the blood of infected individuals. By repurposing a handheld microfluidics-based cell counter (Scepter™), we demonstrate that microfilariae can be identified and quantified using minimal volume of whole blood (20µl) after lysis with 10% saponin. A highly significant correlation (r=0.9182, p<0.0001) was observed between counts obtained by microscopy and those obtained using the Scepter™ study using 20µl of blood with microfilariae of *Brugia malayi*, *Dirofilaria immitis* or *L. loa*. Preliminary proof of concept studies in Cameroon with 20µl of *L. loa* infected human blood (n=30) and experimentally infected baboons (n=4) with a wide range of microfilaria levels demonstrated that the counts obtained by calibrated thick blood smears and those by Scepter™ were highly correlated (r=0.8504, p<0.0001), though at very low levels of microfilaria, there was a loss of sensitivity with the Scepter™. Moreover, the time from blood draw to microfilarial count for the Scepter™ was between 1-2 minutes whereas for the calibrated thick smear the time ranged between 4 hours and 2 weeks. The data suggest that we have a sensitive, rapid, point-of-care and quantitative test to identify individuals with levels of *L. loa* microfilariae that put them at risk for SAEs. In addition, it requires minimal blood volumes, is highly portable, independent of ambient temperature and humidity and provides ease of data storage and accessibility.

1418

GLOBAL RISK MAPS OF THE *LEISHMANIA*SES

David M. Pigott[1], Yves Balard[2], Patrick Bastien[2], Samir Bhatt[1], John S. Brownstein[3], Kirsten Duda[1], Dylan B. George[4], Monica F. Myers[1], Francine Pratlong[2], Simon I. Hay[1]

[1]University of Oxford, Oxford, United Kingdom, [2]Centre National de Référence des Leishmania, Montpellier, France, [3]Harvard Medical School, Boston, MA, United States, [4]Fogarty International Center, Bethesda, MD, United States

The *Leishmania*ses are a collection of complex infections caused by *Leishmania spp.*, ranging from localised cutaneous lesions to forms with visceral complications. Annual incidence is estimated to be around 1.5 million new cases of cutaneous leishmaniasis, and 0.5 million cases of visceral leishmaniasis. The interplay between humans, the Phlebotomine sandfly vectors and reservoir hosts complicates epidemiological understanding as well as control efforts; research has therefore tended to concentrate on solving clinical and epidemiological aspects of the

disease at small spatial scales. This, combined with the comparatively little funding and research attention the leishmaniases garner, has resulted in no attempt to provide a global evidence-based risk map of these diseases. For each sub-national province, an assessment of cutaneous and visceral leishmaniasis was performed incorporating data from the WHO Expert Committee and the Global Infectious Diseases and Epidemiology Network as well as peer-reviewed disease occurrences and reported annual caseloads. These data were used to quantitatively assess certainty of the diseases' presence or absence on a continuous scale. A global database of close to 20,000 geo-positioned data points was collected from peer-reviewed literature using Web of Knowledge and PubMed searches and lab confirmed case data. Using a predictive Boosted Regression Trees modelling approach, separate continuous global risk maps for Cutaneous and Visceral Leishmaniasis were produced. We predict Leishmaniasis risk throughout Central and Southern America, as well as from the Mediterranean Basin to Western China, with other foci in Central and Southern Africa. Climatic and environmental variables were identified as important in defining this distribution. It is hoped that such a map will help inform not only future epidemiological studies but also public health policy directed towards these diseases, allowing improved targeting of specific control efforts with humans, vectors and reservoirs, as well as identify suitable areas for surveillance both active and passive.

1419

GLOBALLY FIRST TIME SYSTEMATICALLY CASE-BASED INTRODUCTION OF LLIN (LONG LASTING INSECTICIDE TREATED NET) AND ITS IMPACT IN KALA-AZAR (VISCERAL LEISHMANIASIS) ELIMINATION IN HYPER ENDEMIC SUB-DISTRICTS OF BANGLADESH

Tanveer A. Choudhury

Medical Officer, Directorate General of Health Services, Ministry of Health and Family Welfare, Bangladesh, Dhaka, Bangladesh

Bangladesh along with Nepal and India is committed to Eliminate Kala-azar (Visceral Leishmaniasis) by 2015. Effective Integrated Vector Management (IVM) is one of the main strategies for Kala azar elimination and LLIN (Long Lasting Insecticide treated Net) is one of the tools for this IVM for preventing human- vector contact. The objective of systemically case based LLINs distribution is to implement the Integrated Vector Management strategy for Elimination of Visceral Leishmaniasis in Hyper Endemic sub-districts of Bangladesh and thus pave the way for elimination status by 2015 and to examine the effectiveness of LLIN tool in reducing the human- vector (Sand fly) contact, and improving awareness building in community for helping in identification of new KA /PKDL (Post Kala-azar Dermal Leishmaniasis) cases through Campaign distribution approach. Total 9494 patient both kala-azar and PKDL, registered since 2008 in 8 hyper endemic sub district of Bangladesh had received the nets. Village-unit approaches were followed. Baseline data was collected and selection of sub-district was done on endemicity criteria and cases were identified from 2008 -- August 2012. A LLIN distribution strategy and Micro plan were developed and also for BCC material (pre and post distribution). Active case search and selection of patient through field visit was done and advocacy meetings were arranged at Sub-district level to create mass awareness on LLINs. Village wise campaigns were arranged for distribution of LLINs among new and old cases. --one LLIN for each patient and extra one for his/her family members. Proper monitoring, evaluation, follow- up program was done by the field managers. Globally practiced for first time, as a tool for Integrated Vector Management (IVM) for Visceral Leishmaniasis Elimination Program, case based LLIN distribution was successfully implemented and can be practiced in other countries. To reduce the transmission of Kala azar from reservoir to Vector, LLIN distribution can play a vital role for PKDL patients. Also lessons were learned that LLIN distribution among KA/KDL cases, with prior campaign for community participation can act as Catch-up strategy for new case identification.

1420

MULTI-SCALE MIGRATION PATTERNS OF *TRIATOMA INFESTANS* IN AN URBAN ENVIRONMENT AND IMPLICATIONS FOR LONG TERM PREVENTION OF CHAGAS DISEASE

Corentin M. Barbu[1], Karthik Sethuraman[1], Jennifer M. Manne[2], Javier E. Quintanilla Calderón[3], Malwina Niemierko[1], Víctor Quispe-Machaca[4], Michael Z. Levy[1]

[1]University of Pennsylvania, Philadelphia, PA, United States, [2]Harvard School of Public Health, Boston, MA, United States, [3]Asociacion Benefica PRISMA, Lima, Peru, [4]Universidad Peruana Cayetano Heredia, Lima, Peru

Arequipa, with close to one million inhabitants, is Peru's second largest city. It is currently undertaking a campaign to control *Triatoma infestans*, the main vector of Chagas disease. *T, infestans* mobility has long been linked to human movement, suggesting that separate vector populations in large, interconnected urban centers such as Arequipa may behave as a large connected population. Treated households could potentially be recolonized by vectors from households that did not participate in the control campaign or by neigborhoods still awaiting treatment. Here, we develop a new spatial model-based methodology to estimate *T, infestans* migration patterns at the city-block, neighborhood, and city level. We apply this method to spatio-temporal infestation data collected during vector control activities. We estimate that an existing infested household will generate a secondary infestation in a completely susceptible population on average every 1.12 years [0.9-1.3]. We find that the rate of dispersal to neighboring city blocks is on the same order of magnitude as longer-distance dispersal and that both of these are much less common than dispersal within a city block. These estimates are compatible with previously observed auto-correlation patterns of infestation showing a strong barrier effect of streets and with genetic diversity patterns observed using microsatellite markers. The relative importance of migration to distant households suggests that propagation of infestation outside a city-block is largely due to passive transport and probably linked to human movements and is much less determined by distance than by active insect dispersal. In the context of low participation rates in the control campaign (60-85%) along with a high infestation prevalence prior to control (10-30%), we discuss the impact of migration on vector surveillance requirements over time and more generally how long-term vector control planning can be based on epidemiological data routinely collected during control efforts.

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SCREENING OF ANTI-INFECTIVES AGAINST *PLASMODIUM* AND KINETOPLASTIDS: A SERVICE FOR THE RESEARCH COMMUNITY

Jessey L. Erath, Grasiella Andriani, Ana Rodriguez

New York University School of Medicine, New York, NY, United States

The Anti-Infectives Screening Core, a non-profit entity created to facilitate early drug development for neglected diseases, takes advantage of the specialized facilities and expertise at NYU Parasitology for testing candidate molecules for parasitic diseases *in vitro* and *in vivo*. Currently, the core tests anti-infectives for four parasitic neglected diseases: Malaria, Chagas Disease, Human African Trypanosomiasis, and Leishmaniasis. Compounds are provided by users and shipped to the core for testing without revealing structures or any IP involvement. We have set up *in vitro* assays that determine the potency of compounds (EC50) for specific parasites: *Trypanosoma cruzi* (intra-host cell amastigotes), *Trypanosoma brucei brucei* (bloodstream forms), *Leishmania amazonensis* (promastigotes, axenic amastigotes or intra-macrophage amastigotes), *L. donovani* (promastigotes), *L. major* (promastigotes), and *Plasmodium falciparum* asexual stages, in addition to quantification of the cytotoxicity (TC50). Each assay contains negative and positive controls and each determination is performed in duplicate. In vivo assays take advantage of transgenic parasites that express luciferase, which allows rapid automated

quantification of infection. Groups of five mice are infected with any of the parasites of study: *T. cruzi, T. brucei brucei, L. amazonensis* and *P. berghei* (liver, blood stage, gametocyte or mosquito transmission). When infection has progressed, mice are imaged to quantify the luminescence signal, which is proportional to the parasite load (baseline infection level). Treatment with the test compounds begins, normally administered via i.p. injection or oral gavage. One day after the last treatment dose, mice are imaged again to determine the level of infection. Results are expressed as the ratio of infection at the end of treatment versus the base infection for each animal. Testing includes a negative control and positive control groups with a well-known drug for each disease. http://ocs.med.nyu.edu/anti-infectives-screening

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ESTABLISHING BIOMARKERS OF *LEISHMANIA DONOVANI* INFECTION: POTENTIAL ENDPOINTS FOR VACCINE TRIALS

Aarthy C. Vallur[1], Caroline Reinhart[1], Yeung Tutterrow[1], Dinesh Mondal[2], Khondaker R. Bhasker[2], Malcolm S. Duthie[1], Rhea N. Coler[1], Steven G. Reed[1]

[1]Infectious Diseases Research Institute, Seattle, WA, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

Visceral leishmaniasis in the Indian sub-continent has been targeted for elimination by 2015. The realization of this goal is dependent upon identifying early infection in asymptomatic individuals, who present no overt symptoms and are potential reservoirs for spreading infection and developing disease. We studied asymptomatic individuals in the *Leishmania donovani*-hyper endemic Mymensingh district in Bangladesh over a period of 24 months to define the natural dynamics of *Leishmania*-specific antibodies and DNA and reveal their utility as biomarkers of infection. Samples were analyzed by DAT; *L. donovani* whole cell lysate and rk39 ELISA; and quantitative PCR. Serological tests indicated the sustained presence of antibodies at study intake and at a 12-month follow up interval. By DAT, 57% tested positive at both time points while by ELISA, 82% and 89% were positive at baseline and 75% and 92% tested positive at 12 month follow-up to *L. donovani* whole cell lysate and rk39, respectively. In contrast, though 84% tested positive by PCR at baseline, only 28% remained positive at follow-up. During the course of the study, 3 of the 56 study subjects developed symptomatic VL, with each consistently testing positive for antibodies or nucleic acids. Our results reflect the transient nature of asymptomatic *L. donovani* infection in this endemic area. Used together, the presence of *Leishmania*-specific antibodies and nucleic acids can predict individuals who are at the highest risk of progression to disease and thus will gain most from intervention. Based on our results, we suggest means by which tests for *Leishmania*-specific antibodies and circulating *Leishmania* DNA can be used in active surveillance of endemic areas. We conclude that these bio markers will be valuable in identifying populations for vaccine trials as well as serve as end points to evaluate the effectiveness of the trials.

1423

NOVEL NANOTECHNOLOGY TO CONCENTRATE AND PRESERVE *TRYPANOSOMA CRUZI* ANTIGENS IN URINE FOR EARLY DIAGNOSIS OF REACTIVATION OF CHAGAS DISEASE IN PATIENTS CO-INFECTED WITH HIV VIRUS

Yagahira E. Castro[1], Robert H. Gilman[2], Melissa Reimer[3], Carolina Carolina Mejia[1], Eduardo Valencia Eduardo[1], Lance Lance Liotta[4]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Johns Hopkins University, Baltimore, MD, United States, [3]University of South Alabama, Alabama, AL, United States, [4]George Mason University, Manassas, VA, United States

We developed harvesting nano-porous particles to capture, concentrate and preserve *Trypanosoma cruzi* antigens in urine of HIV/*T. cruzi* patients. Diagnosis of reactivation of chronic Chagas disease in HIV/*T. cruzi* patients is based on detection of *parasitemia* by micromethod but lacks sensitivity. Antigenuria has been shown to be correlated with *parasitemia*, but has also low sensitivity. Poly N-isopropylacrylamide (NIPAm) based particles are functionalized with chemical baits (trypan blue, TB) that capture antigens with high affinity (KD<10-12 M) within minutes, antigens captured can be eluted in a small volume yielding a concentration factor that is the ratio between the initial volume of urine and the final elution volume. In this study, model urine samples were incubated with poly(NIPAm)/TB particles. Antigens eluted from the particles were detected by Western Blot using a polyclonal antibody against *T. cruzi* H49 antigen. Nano-porous particles increased the sensitivity of antigenuria by *T. cruzi* more than 100 fold (detection limit was 0.8 ng/ml with particle treatment compared to 100 ng/ml without particle treatment). This assay was applied to a cohort of HIV/*T. cruzi* co-infected patients (N=39, 20 *T. cruzi* positive and 19 *T. cruzi* negative). Sensitivity of antigenuria in the particle-concentrated urines was 100% (2/2), 90% (9/10) and 80% (16/20) compared to micromethod, PCR and ELISA, respectively. The specificity was 100%. Positive results of antigenuria were correlated to high levels of *parasitemia* ($p<0.05$). Particle-sequestered *T. cruzi* H49 antigen was protected from enzymatic degradation by trypsin digestion and in urine over seven days at room temperature, showing that particles protected urinary antigens from degradation. Nano-porous particles effectively concentrated *T. cruzi* antigens in urine. Nanotechnology-enhanced antigenuria test could be an early predictor of reactivation and can be adapted for monitoring HIV/ *T. cruzi* co-infected patients. Particle integration in urine collection is envisioned for sample handling and shipment at room temperature.

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AN7973: A NOVEL OXOBOROLE FOR THE TREATMENT OF AFRICAN ANIMAL TRYPANOSOMIASIS

Yvonne R. Freund[1], Tsutomu Akama[1], Kirsten Gillingwater[2], Virginia Sanders[1], Wei Bu[1], Grant B. Napier[3], Tim Rowan[3], Michael Witty[3], Bakela Nare[4], Jacob J. Plattner[1], Eric Easom[1]

[1]Anacor Pharmaceuticals, Inc., Palo Alto, CA, United States, [2]Swiss Tropical and Public Health Institute, Basel, Switzerland, [3]Global Alliance for Livestock and Veterinary Medicine, Edinburgh, United Kingdom, [4]SCYNEXIS, Inc., Research Triangle Park, NC, United States

African animal trypanosomiasis (AAT) is a parasitic disease caused by tsetse fly-transmitted trypanosomes, which include *Trypanosoma congolense (T.c.), T. brucei brucei (T.b.b.),* and *T. vivax*. AAT results in serious economic losses in livestock due to reduced productivity from anemia, emaciation and fever. AN7973 is a novel boron-containing molecule that demonstrates excellent potency against *T. congolense in vitro* as well as *in vivo* efficacy against *T.c* infection in mice, goats, and cattle. AN7973 has an IC_{50} of 0.057 μM and 0.098 μM against *T. c.*, and *T. b. b.*, respectively. Compound wash-out experiments demonstrate that a 10 h exposure to AN7973 results in irreversible killing at concentrations as low as 1.25 μg/mL (3.3 μM). By 24 h, 99% of the parasites were killed at concentrations of 0.15 μg/mL (0.40 μM), demonstrating the trypanocidality of AN7973. PK studies were performed in mouse, rat, dog, and cattle. Intramuscular (IM) injection of 5 mg/kg in cattle resulted in a C_{max} of 2.05 μg/mL, $AUC_{(0-24)}$ of 89.1 h*μg/mL and a terminal half life of 22.6 h. AN7973 was tested for *in vivo* efficacy in a murine model of *T.c.* infection. A single dose of 10 mg/kg showed 100% cure, 60 days after treatment. In a goat model of *T.c.* infection, 10 mg/kg by IM injection demonstrated 100% cure at Day 100. In a cattle efficacy study, using a diminazene- and isometamidium-resistant strain of *T.c.,* a single IM dose of 10 mg/kg or 2 doses of 5 mg/kg, 24 h apart, also demonstrated 100% survival at 100 days post-treatment, reflecting complete cure. The plasma concentration in the cattle efficacy study at 1 x 10 mg/kg was 1.93 μg/mL at t = 24 h, well above the concentrations necessary for cidality. The plasma exposure (AUC_{0-24h}) was determined to be 46.1 h*μg/mL. AN7973 was well tolerated in a cattle safety study during which AN7973 was dosed at 30mg/kg three times with

2 weeks separation between doses. In summary, AN7973 demonstrates excellent efficacy against *T.c.* in the target animal, and shows promise as a novel chemical entity for treatment of AAT.

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VILLAGE-LEVEL CHARACTERISTICS ASSOCIATED WITH SPATIAL DISTRIBUTIONS OF MALARIA-INFECTED INDIVIDUALS IN AN AREA OF SOUTHERN ZAMBIA RECEIVING MASS SCREENING AND TREATMENT

David A. Larsen[1], John M. Miller[2], Joseph Keating[3], Joshua Yukich[3], Jeffrey Shaffer[4], Busiku Hamainza[2], Kafula Silumbe[5], Thomas Eisele[3]

[1]Department of Public Health, Food Studies and Nutrition, Syracuse University, Syracuse, NY, United States, [2]Malaria Control and Evaluation Partnership in Africa (MACEPA), a program at PATH, Lusaka, Zambia, [3]Center for Applied Malaria Research and Evaluation, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States, [4]Department of Biostatistics, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States, [5]Malaria Control and Evaluation Partnership in Africa (MACEPA), a program at PATH, New Orleans, LA, United States

Malaria clusters across space and time; malaria interventions may be targeted to maximize the efficiency of scarce resources. However, how to target malaria interventions toward small foci of transmission is not well understood. An ongoing mass screening and treatment (MSAT) intervention in southern Zambia has provided census data at 4 different time from Dec 2011 – Nov 2012. The difference in K function, which assesses spatial regularity or clustering compared to randomness at any distance, was used to assess the spatial distribution of malaria-infected individuals observed during each census. Individuals with a malaria infection clustered within households at all prevalence levels and month of the census. Beyond the household, individuals with malaria infections were distributed differently in space depending both on village parasite prevalence and month of the census. Because the spatial distribution of malaria-infected individuals varied by prevalence level, malaria parasite prevalence aggregated to the village level was then modeled to determine factors that may explain the differing spatial distributions. A linear mixed effects model including altitude, enhanced vegetation index, nighttime temperature, round of MSAT (categorized as round 1-4) and the topographical position index (whether a village was located in a valley, ridge, plain, or slope) accounted for 82.5% of the variation in village malaria parasite prevalence. This technique also revealed that an increase in altitude of 100m was associated with an absolute 2.5% decrease in village parasite prevalence ($p < 0.001$). An increase from 0 (dry, brown foliage) to 1 (green dense foliage) in the enhanced vegetation index was associated with an absolute 44% increase in village parasite prevalence ($p < 0.001$). A 1-degree increase in nighttime temperature was associated with an absolute 0.4% increase in village parasite prevalence ($p < 0.01$). Multiple rounds of the MSAT intervention were also associated with decreased village malaria parasite prevalence; 3 rounds were associated with an absolute 9.6% decrease (relative 38.6%) from the baseline round ($p < 0.001$). Varying spatial distributions of malaria-infected individuals appear to be driven by vector abundance and gametocyte prevalence in the population. The ability to clearly delineate village malaria prevalence may assist in developing mechanisms for focused interventions to optimize their effectiveness.

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RAPID SCALE-UP OF LONG-LASTING INSECTICIDAL NETS ASSOCIATED WITH A DECREASE IN SEVERE MALARIA INCIDENCE AND AN UPWARD SHIFT IN THE MEAN AGE OF SEVERE CASES AMONG CHILDREN IN LUANGWA DISTRICT ZAMBIA

Thomas P. Eisele[1], Adam Bennett[1], David A. Larsen[2], Benjamin Kalayjian[3], Josh Yukich[1], Joseph Keating[1], John M. Miller[4], Busiku Hamianza[5], Richard W. Steketee[6]

[1]Center for Applied Malaria Research and Evaluation, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States, [2]Syracuse University, Syracuse, NY, United States, [3]Tulane University School of Medicine, New Orleans, LA, United States, [4]PATH-MACEPA, Lusaka, Zambia, [5]National Malaria Control Centre, Lusaka, Zambia, [6]PATH-MACEPA, Seattle, WA, United States

Long-lasting insecticidal nets (LLINs) have been shown to reduce malaria transmission by as much as 90% with concomitant reduction in malaria incidence and all-cause child mortality in the African Africa. Ecological data have also shown the mean age of severe malaria to shift from younger to older children as transmission decreases in these settings. In late 2005 and 2006 16,100 LLINs were distributed free of charge to all households (approximately 4,000)in the Luangwa District of Zambia, resulting in rapidly achieving high household coverage of LLINs (73%), from very low coverage prior to 2005. We assessed trends in the mean age of children under 10 years old admitted to the two hospitals serving Luangwa District from January 2003 through August 2009. A difference-in-difference analytic approach was used in a linear regression model to assess change in the mean age of reported child hospital admissions (primary outcome), categorized as malaria or non-malaria diagnosis, before and after LLIN scale-up (before and after January 2007), while controlling for hospital, malaria transmission season, and lagged monthly vegetation index and mean temperature. This approach allowed us to assess the relative change in the mean age of reported in-patient severe malaria cases compared to all hospitalized admissions due to causes other than malaria over this time period. Total reported in-patient admissions among children under 10 years old decreased from 63.8 per 1,000 in 2003 to 41.2 per 1,000 in 2009. Reported in-patient severe malaria admissions decreased from 41.7 to 21.5 per 1,000 population over this same period; severe malaria as the cause of admission decreased from 64.6% in 2003 to 51.6% in 2009. The mean age of non-malaria admissions stayed relatively constant over the observation period at 23.9 months, while the mean age of severe-malaria admissions increased from 17.1 months prior to LLIN scale-up (before 2007) to 23.5 months post LLIN scale-up (coefficient for malaria diagnosis X pre-post LLIN period interaction term = 0.47 (in years); p-value <0.001). Results suggest a decline in reported severe malaria admissions following LLIN scale-up, with a coinciding upward shift in the mean age of severe malaria admissions. These results suggest that the rapid LLIN scale-up in Luangwa district was associated with a marked decrease in malaria transmission, severe illness, and modifications in the age distribution of those afflicted.

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SUSTAINED DECLINING BURDEN OF MALARIA AT COMMUNITY LEVEL IN NORTHEASTERN TANZANIA

Acleus S. Rutta, Filbert Francis, Bruno P. Mmbando, Deus S. Ishengoma, Samuel Sembuche, Ezekiel K. Malecela, Johari Y. Sadi, Mathias L. Kamugisha, **Martha M. Lemnge**

National Institute for Medical Research, Tanga, United Republic of Tanzania

The reported decline of malaria in most parts of Tanzania has some implication on accuracy of malaria diagnosis and management, especially following the introduction of expensive artemisinin combination therapy (ACT) with artemether/lumefantrine (ALu). Traditionally, fever has been the back-bone of malaria case management; but with declining malaria and

introduction of expensive ACTs, this approach poses a major challenge. In our previous and ongoing malaria passive case detection in 4 villages of Korogwe, northeastern Tanzania, we demonstrated that provision of early diagnosis and treatment of malaria by community owned resource persons (CORPs) using rapid diagnostic tests (RDTs) and ALu is an effective strategy for malaria control. We now provide updates on sustained impact of these interventions on malaria in communities where the transmission has significantly declined. In 2006, individuals with history of fever within 24 hours or fever (≥37.5°C) at presentation were presumptively treated with sulphadoxine/pyrimethamine. Between 2007 and 2012, individuals aged 5 years and above with positive RDTs were treated with ALu while under-fives were treated irrespective of RDT results. A total of 18,981 cases were attended and 17.2% were positive for malaria parasites by microscopy. Malaria prevalence and incidence decreased across the years, from 34.6% to <1% and 235/1000 to <8/1000 person years at risk for 2007 and 2012, respectively. The highest incidence of malaria shifted from children aged 5-9 years to individuals aged 10-19 years from 2009. Despite these changes, fever prevalence remained high at >40.0% in under-fives and >20.0% among individuals aged 5 years and above. The significant reduction in malaria prevalence and incidence observed might be attributed to different interventions including early diagnosis and prompt treatment through CORPs strategy. Studies to investigate causes of fevers other than malaria are recommended for better case management. The current remarkable and sustained decline in malaria suggests that these areas might be moving from control to pre-elimination levels.

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RESERVOIRS OF ASYMPTOMATIC MALARIA IN MALAWI: RESULTS OF TWO CROSS-SECTIONAL STUDIES

Jenny A. Walldorf[1], Lauren M. Cohee[1], Jacqueline Fiore[2], Andy Bauleni[3], Jenna E. Coalson[4], Themba Phiri[3], Don Mathanga[3], Clarissa Valim[5], Atupele P. Kapito-Tembo[3], Terrie E. Taylor[2], Miriam K. Laufer[1]

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

Malaria surveillance in endemic countries typically focuses on young children who are at highest risk of malaria morbidity and mortality. As we develop strategies to eliminate malaria, it is critical to expand our understanding of sources of malaria transmission. The Malawi International Center for Excellence in Malaria Research conducted cross-sectional surveys in the 2012 rainy and dry seasons in three transmission settings in southern Malawi with the goal of estimating prevalence of asymptomatic malaria infection and assessing risk factors for asymptomatic *parasitemia* in each setting. Districts were selected to represent urban/low (Blantyre City), rural/high (Chikhwawa), and semi-rural/mountainous (Thyolo) malaria transmission. We randomly selected 30 households in 10 enumeration areas in each district. Demographic, malaria intervention, and current health status data were collected through household interviews; blood samples were obtained from all individuals over six months of age. Among 5099 individuals with smear results in Blantyre, Chikhwawa, and Thyolo, total parasite prevalence was 11.7%, 13.1%, 11.0% in rainy and 3.9%, 17.4%, 9.4% in dry seasons respectively. Asymptomatic *parasitemia* represented 46.2%, 41.7%, 49.3% and 76.7%, 69.5%, 79.0% of total parasite prevalence in the two seasons, respectively. In multinomial regression using aparasitemic individuals and age 6-59 months as reference groups and controlling for district, individual net use and indoor residual spraying, ages 5-15 years was strongly associated with asymptomatic *parasitemia* in the rainy season (Odds ratio (OR) = 6.7, [95% Confidence interval (CI): 3.3, 13.7]) and also in the dry season (OR = 1.5 [95% CI: 1.1, 2.2]). Age >15 years was not significantly associated with asymptomatic *parasitemia* in the rainy season but was protective (OR = 0.64, [95% CI: 0.45, 0.92]) in the dry season. In Malawi and potentially in other endemic settings, school age children represent important reservoirs of asymptomatic infection and should be targeted for interventions to interrupt transmission.

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MICROEPIDEMIOLOGY OF SUB-MICROSCOPIC *PLASMODIUM FALCIPARUM* INFECTION: IMPLICATIONS FOR DETECTION OF HOTSPOTS WITH IMPERFECT DIAGNOSTICS

Jacklin Mosha[1], **Hugh Sturrock**[2], Bryan Greenhouse[2], Brian Greenwood[1], Daniel Chandramohan[1], Colin Sutherland[1], Drakeley Chris[1], Sharan Atwal[1], Nahla Gadalla[1], Gibson Kibiki[3], Teun Bousema[1], Roly Gosling[2]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]University of California San Francisco, San Francisco, CA, United States, [3]Kilimanjaro Christian Medical Centre, Moshi, United Republic of Tanzania

At the local level, malaria transmission clusters in hotspots, which may be a single household or group of households that experience higher than average exposure to infectious mosquitoes. Active case detection (ACD), often relying on rapid diagnostic tests (RDTs) for mass screen and treat campaigns, has been proposed as a method to detect and treat individuals in hotspots. Here we used data from a cross sectional survey conducted in north-western Tanzania to examine the spatial distribution of *Plasmodium falciparum* to establish whether RDTs are likely to have sufficient sensitivity to target ACD interventions aimed at reducing transmission. Dried blood spots were collected from all consenting individuals from four villages in a single ward during a survey conducted between August and November 2010. These were analyzed by PCR for the presence of *P. falciparum*, with the parasite density of positive samples being estimated by quantitative PCR. Household exposure was estimated using distance-weighted PCR prevalence of infection. Results showed that mean distance-weighted PCR prevalence per household was 34.5% (range 0 - 94.7%). Infection density was highest in children 5-10 years old and lowest in those >40 years old. Infection density was negatively associated with transmission intensity with the odds of an infection being sub-microscopic increasing with household exposure (OR 1.09 per 1% increase in exposure, p<0.001). This relationship, which is potentially explained by exposure-related immunity, suggests that RDTs and microscopy have the lowest sensitivity in transmission hotspots. Simulations of different targeted mass drug administration (tMDA) strategies showed that treating all individuals in households where RDT prevalence was above 20% increased the number of infections that would have been treated from 43% to 55%, however, 45% of infections remained untreated. Even using a single RDT positive as a trigger for household MDA resulted in around 35% of infections remaining untreated. Taken together, these results suggest that community wide MDA, instead of screen and treat strategies, may be needed to successfully treat the asymptomatic, submicroscopic parasite reservoir and reduce transmission in similar settings.

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OPERATIONAL APPROACHES FOR DETECTING FOCI OF MALARIA INFECTION: HOW DO SCHOOL AND HEALTH FACILITY SURVEYS COMPARE AGAINST A COMMUNITY-BASED APPROACH

Gillian H. Stresman[1], Jennifer Stevenson[1], Chrispin Owaga[2], Elizabeth Marube[2], Wycliffe Odongo[2], Shehu Shaggari[2], Teun Bousema[1], Chris Drakeley[1], Jonathan Cox[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Kenya Medical Research Institute, Kisumu, Kenya

There is increasing evidence of heterogeneity manifested through presence of foci of malaria infection. Tailoring interventions to reflect this heterogeneity is likely to bring benefits in terms of their impact and cost effectiveness. For such a targeted approach to be effective in the long term, strategies are needed to enable local malaria control teams to reliably identify and target the true foci of infection in the community.

In July 2010, 4987 children were tested for malaria across 46 schools in Rachuonyo South district in western Kenya and the compounds of 4888 children (98%) were geolocated. Two surveys were conducted in 5 health facilities in the same area (Oct 2011, July 2012) with a combined total of 3034 people tested for malaria. Of the participants sampled, spatial coordinates of the compound were obtained for 30% of the participants. All participants sampled at school and health facilities were tested for malaria by rapid diagnostic test and samples from all surveys were assessed for antibody response to *Plasmodium falciparum* AMA1 and MSP1. The results were compared to foci of infection identified during a community cross-sectional survey of 17506 individuals. Preliminary results indicate that if positive for malaria by RDT or serology, participants had twice the odds of residing in foci of infection (p<0.0001). Seropositivity in schools surveys had a sensitivity of 64.1% in identifying children that reside in known foci whereas RDT results obtained during the health facility surveys were 86.8% specific in identifying children that do not reside in known foci of infection. Results indicate that school and health facility surveys may provide an alternative approach to detect foci of infection in the community. However, the definition of foci of malaria infection from both an operational and academic perspective is in need of further discussion.

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USING SEROLOGICAL MARKERS FOR ESTIMATING MALARIA TRANSMISSION INTENSITY AND ASSESSING INTERVENTION EFFICACY IN WESTERN KENYA

Jacklyn Wong[1], Mary J. Hamel[1], Chris J. Drakeley[2], Simon K. Kariuki[3], Ya Ping Shi[1], John M. Vulule[3], John E. Gimnig[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]Kenya Medical Research Institute, Kisumu, Kenya

Accurate measurement of local malaria transmission is critical for evaluating control interventions. Serological conversion rates (SCRs) have been used to estimate the force of malaria infection in populations. In low to moderate transmission settings, systematic reductions in incidence (e.g., due to effective interventions) can be measured through a single retrospective serological survey. Our objective was to validate, in a malaria hyperendemic region, the accuracy of serological markers for 1) estimating transmission intensity and 2) retrospectively detecting a decline in incidence. Asembo, western Kenya, is an area that experiences high, perennial malaria transmission. From 1997-1999, Asembo was the site of a community-wide insecticide-treated bed net (ITN) trial that reduced malaria transmission by 90%. Serological samples collected pre-ITN (1994) and post-ITN (2009) were tested by indirect ELISA for antibodies against *Plasmodium falciparum* circumsporozoite protein (CSP), merozoite surface protein-1 (MSP-1), and apical membrane antigen-1 (AMA-1). Age-specific seroprevalence data were fitted to catalytic conversion models to estimate SCRs for 1994 and 2009. Post-ITN (2009) age-seroprevalence curves were also examined for tiered trends to denote when transmission declined. Between 1994 and 2009, SCRs for CSP, MSP-1, and AMA-1 fell by 50%, 25%, and 49%, respectively. SCRs corresponded closely with entomological inoculation rates, which dropped from >100 to 10 infectious bites/person-year during this 15 year period. Post-ITN (2009) SCRs were uniform across all ages rather than tiered; older age groups born before the trial did not exhibit higher SCRs than young age groups born after. We conclude that serological markers provide reliable estimates of malaria transmission intensity near the time of sample collection. Because we were unable to pinpoint when the drop in transmission occurred, however, this model did not appear to be accurate in malaria hyperendemic areas for retrospective reconstruction of historical trends associated with control interventions.

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INNATE LYMPHOID CELL POPULATIONS DRIVE THE TH2 IMMUNE RESPONSE TO *LITOMOSOIDES SIGMADONTIS* IN BALB/C MICE

Alexis Boyd[1], Kristin E. Killoran[2], Edward Mitre[2], Thomas B. Nutman[1]

[1]National Institutes of Health, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, [2]Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda, MD, United States

A group of innate cells, termed nuocytes, multipotent progenitor (MPP) cells or innate helper cells (IHC) [collectively referred to as innate lymphoid cells (ILCs)] have been identified at barrier surfaces and are involved in propagating a Th2-type response following infection with intestinal helminths in mice. *Litomosoides sigmodontis* (Ls) is a filarial parasite of rodents that lives in the pleural cavity and develops a Th2-response at patency (when microfilariae are produced) at day 42 post infection (p.i.). To assess whether the pleural cavity also utilizes ILCs to drive a Th2 response, Balb/c mice were infected with 40 infective stage larvae (L3) of Ls and the frequencies of ILC subpopulations were determined by multiparameter flow cytometry of cells isolated from the spleen and pooled samples from the pleural cavity on days 5, 14, 36, 42, and 60 p.i. ILCs were defined as lineage-/cKit+ and were further divided by Sca1+ (MPPs), Sca1-/CD90.2+/CD44+ (IHCs) and Sca1+/CD90.2+/CD44+/ST2+ cells (nuocytes). Each of these ILC subpopulations was identified in the spleen and pleural cavity of infected and uninfected mice. Two of the 3 ILC subpopulations were significantly expanded in the spleen at day 42 p.i., compared to uninfected matched controls (nuocytes: p= 0.021, IHC: p=0.044). In the pleural cavity, there was an increased frequency of MPPs and nuocytes at day 36 and day 42 p.i. compared to controls and an increase of IHCs at day 42 p.i. The cellular infiltrate in the pleural cavity during infection showed that neutrophils (22-fold day 36, 68-fold day 42), eosinophils (23-fold day 36, 9-fold day 42) and macrophages (26-fold day 36, 6-fold day 42) increased markedly at day 36 to day 42 p.i. This increase was accompanied by an increase from baseline in the levels of IL-4 (16-fold day 36, 34-fold day 42), IL-5 (144-fold day 36, 218-fold day 42), IL-13 (167-fold day 36, 3-fold day 42) and IL-10 (1.5-fold day 36, 27-fold day 42) in the pleural lavage fluid and by increases in plasma IL-5 levels (p=0.0097 day 36, p=0.0570 day 42) and levels of IgE (p-value=0.0079 day 42) and IgG1 (p-value=0.0079 day 42) antibodies. These data confirm the induction of a Th2-dominant response both locally and systemically by Ls and suggest that ILCs may be a major contributing factor. Since these ILCs are typically induced by barrier cell-expressed IL-25 and IL-33, the cellular sources of these cytokines and their influence on ILC/Th2 cell induction are currently under study.

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M2A MACROPHAGES ARE NECESSARY AND SUFFICIENT TO MEDIATE EOSINOPHIL-DEPENDENT IMMUNITY TO FILARIAL HELMINTH INFECTION

Joseph D. Turner[1], Ana de Castro Guimaraes[1], Alice Halliday[1], Kathryn J. Else[2], Nico van Rooijen[3], Andrew Steven[1], Darren Cook[1], Mark J. Taylor[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]University of Manchester, Manchester, United Kingdom, [3]Vrije Universiteit, VUMC, Amsterdam, Netherlands

Eosinophils are effector cells in the immune control of tissue dwelling helminths. Whilst eosinophil responses are induced by Th2 adaptive immunity, it is not known how eosinophils are instructed to home from the blood to target migratory stages of parasites. Here we provide evidence from an experimental model of filarial infection (*Brugia malayi* mouse model) that eosinophils are a crucial component of the anti-filarial response that limits establishment of infectious larvae. *B. malayi* infectious larvae also induce M2a macrophage activation (alternative activation)

of tissue resident macrophages, which is further pronounced following vaccination with heat-killed larvae. Absence of a functional interleukin 4 receptor α chain (IL-4Rα) leads to failure of M2a activation, impaired eosinophil recruitment and susceptibility to *B. malayi* establishment to the adult phase. To test the functional relevance of M2a development in the eosinophil larvicidal response, we undertook targeted depletion of macrophages by clodronate liposome (CL) treatment. CL-treatment rendered mice highly susceptible to infection with associated impaired eosinophil recruitment. Add-back of purified M2a into CL-treated WT mice restored both M2a expansion and eosinophil influx. Th2 responses were intact in CL-treated WT mice, suggesting that M2a development directly regulated eosinophil recruitment at the infection site. Consistent with this, an increase in CCL11 transcripts from immune cells derived from the infection site of WT but not IL-4Rα-/- mice was apparent. We therefore tested the direct role of M2a in eosinophil regulation and parasite killing by adoptively transferring purified WT M2a into susceptible severe combined immune-deficient mice (SCID). WT M2a SCID recipients induced a rapid eosinophil response and killing of infectious larvae. In a complementary approach, supplying an exogenous source of IL-4 to condition resident macrophages toward an M2a phenotype at the point of infection rendered SCID mice more resistant to larval establishment. Thus we conclude that M2a conditioning via IL-4Rα is both necessary and sufficient in the absence of additional adaptive immune activation to induce resistance to filarial infection via larvicidal eosinophil recruitment.

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ALTERNATIVELY ACTIVATED MACROPHAGES (AAM) IN *SCHISTOSOMA MANSONI* LIVER GRANULOMAS ARE DERIVED FROM MONOCYTES AND ARE PHENOTYPICALLY AND FUNCTIONALLY DISTINCT FROM AAM DERIVED FROM TISSUE MACROPHAGES INDUCED BY *LITOMOSOIDES SIGMODONTIS* INFECTION

P'ng Loke, Natasha M. Girgis, Uma M. Gundra, Lauren N. Ward, Kirsten E. Wiens, Mynthia Cabrera, Ute Frevert

New York University School of Medicine, New York, NY, United States

Alternatively activated macrophages (AAM) are induced by helminth infections. We investigated the origins of AAM found in the liver granulomas of mice infected with *Schistosoma mansoni*. CX3CR1GFP/+ mice were used to track monocytes and AAM through a combination of intravital microscopy and flow cytometry. GFP+ monocytes in the liver sinusoids arrest upon encountering parasite eggs in the vessels. GFP+ cells with macrophage-like morphology accumulate around the eggs, are incorporated into hepatic granulomas and express markers of AAM. To determine if Ly6Clow or Ly6Chigh monocytes serve as AAM precursors, we transferred pure populations of these cells from CX3CR1GFP/+ mice into infected congenic mice. Ly6Chigh monocytes extravasated into the tissue more efficiently and upregulate PD-L2 suggesting that they are the source of AAM during *S. mansoni* infection. However, when transferred Ly6Chigh monocytes extravasated into the tissue they became Ly6Clow, suggesting that Ly6Chigh monocytes may transition through a Ly6Clow state when differentiating into AAM. In addition to monocytes, AAM can also be derived from tissue resident macrophages that proliferate during Litomosoides sigmodontis infection. AAM derived from these different sources may be phenotypically or functionally distinct. We find that while both monocyte and tissue derived AAM express high levels of ARG1, YM1/CHI3L3 and FIZZ1/RELMA, tissue derived AAM expressed high levels of F480, but low levels of MR1 and PDL2. In contrast, monocyte-derived AAM were F480int and expressed high levels of MR1 and PDL2. Monocyte-derived AAM upregulate the enzyme RALDH2, have high levels of Aldefluor activity indicating the production of retinoic acid (RA), whereas tissue derived AAM do not. Consistent with RA production, only monocyte derived AAM can promote the differentiation of FoxP3+ CD4+ cells when used to stimulate naïve CD4+ cells. Therefore, monocyte derived and tissue derived AAM are phenotypically and functionally distinct.

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MOLECULAR CLONING AND CHARACTERIZATION OF NOVEL GLUTAMATE-GATED CHLORIDE CHANNEL SUBUNITS FROM *SCHISTOSOMA MANSONI*

Vanessa Dufour[1], Claudia Wever[2], Conor R. Caffrey[3], Robin N. Beech[1], Paula Ribeiro[1], Joseph A. Dent[2], Timothy G. Geary[1]

[1]McGill University, Sainte-Anne-de-Bellevue, QC, Canada, [2]McGill University, Montreal, QC, Canada, [3]University of California San Francisco, San Francisco, CA, United States

Neuronal receptors of schistosomes are attractive targets for drug development because these parasites depend entirely on neuronal modulation to control functions vital to their survival and reproduction. Cys-loop ligand-gated ion channels (LGIC) are proven drug targets in nematodes and arthropods, but are poorly characterized in flatworms. We have previously cloned 3 glutamate-gated chloride channel (GluCl) subunits from *Schistosoma mansoni* (Sm), and characterized them by two-electrode voltage clamp (TEVC) in Xenopus oocytes. Concentration-response relationships revealed that the SmGluCl receptors affinity for glutamate is among the highest reported for GluCl to date, with EC50 values of 6.87- 26.28 μM. In addition, TEVC showed that SmGluCl receptors are insensitive to ivermectin (IVM), indicating that they do not belong to the highly IVM-sensitive GluClα subtype group. These SmGluCl subunits appear to be the only non-acetylcholine Cys-loop LGICs found in *S. mansoni*. Phylogenetic analyses suggested that they belong to a novel clade of flatworm GluCls, which also includes putative genes from other trematodes and cestodes. This flatworm GluCl clade is evolutionarily distinct from the nematode-arthropod and mollusc GluCl clades, and from all GABA receptors. Using confocal microscopy, we showed that SmGluCls are distributed throughout the central and peripheral nervous systems of *S. mansoni*. Further work is in progress to provide a detailed description of SmGluCl distribution in males, females, cercaria and somules. Finally, we have initiated RNAi-based functional studies to assess the roles played by SmGluCls in schistosomes. Altogether, these results provide the first molecular evidence showing the contribution of GluCl receptors to L-glutamate signaling in *S. mansoni*, an unprecedented finding in flatworms. This project has uncovered a completely new aspect of neuronal modulation in flatworms, and brings attention to very appealing new anthelmintic targets which could be used to address the urgent need for new chemotherapeutic options for schistosomiasis.

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IGE ANTI-SJ6-8 ANTIBODIES PREDICT RESISTANCE TO REINFECTION WITH *SCHISTOSOMA JAPONICUM*

Hai-Wei Wu[1], Sunthron Pond-Tor[1], Mario Jiz[2], Luz Acosta[2], Dipak K. Raj[1], Grant Jolly[1], Jennifer F. Friedman[1], Jonathan D. Kurtis[1]

[1]Rhode Island Hospital, Providence, RI, United States, [2]Research Institute of Tropical Medicine, Manila, Philippines

Our goal is to discover novel vaccine candidates for schistosomiasis japonica by identifying the parasite targets of naturally acquired protective human antibodies. We applied our differential, whole proteome screening method using plasma and epidemiologic data from a longitudinal treatment-reinfection study conducted in Leyte, The Philippines to identify new *Schistosoma japonicum* antigens associated with resistance. Individuals in our cohort (age 8-30 yrs, n=616) were *S. japonicum* infected at baseline, treated with praziquantel and followed with quarterly stool examination for 12 months. We pooled plasma from 10 resistant (RP) and 10 susceptible (SP) individuals, with careful matching for potential confounders, and performed differential screening experiments using an *S. japonicum* adult worm cDNA expression library. We screened 500,000 clones and identified Sj6-8, a 25 kDa hypothetical protein with an IG domain that is uniquely recognized by antibodies in RP but not SP. We have expressed and purified the immuno-relevant region (aa 20-176) in *E. coli* and designated the protein rSj6-8A. Rabbit anti-Sj6-8A recognized a 75 kDa band in adult worm excretory-secretory products and localized

Sj6-8 to the exofacial surface of the tegument and gastrodermis of adult worms by confocal immunofluorescence analysis and immunogold electron microscopy. We developed a bead-based assay to measure anti-rSj6-8A antibody levels in the entire cohort of volunteers. In repeated measures models, individuals with anti-rSj6-8A IgE levels in the upper quartile (n=140) had 58% lower intensity of reinfection measured 12 months after treatment than individuals with anti-rSj6-8A IgE levels in the lowest quartile (n=140, $P < 0.001$) after adjusting for potential confounders including directly observed water contact, village, age, sex, and baseline intensity of infection. Together, these results validate our field-to-lab-to-field based strategy for the rational identification of vaccine candidates and support Sj6-8 as a novel vaccine candidate for schistosomiasis japonica.

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IFN-Γ ELISPOT RESPONSES AGAINST WHOLE SPOROZOITES AND PF ANTIGENS IN VOLUNTEERS IMMUNIZED WITH PROTECTIVE PFSPZ MALARIA VACCINE

Martha Sedegah[1], Robert Seder[2], Jun Huang[1], Harini Ganeshan[1], Maria Belmonte[1], Steve Abot[1], Arnel Belmonte[1], LeeJah Chang[3], Mary Enama[2], Eric James[4], Peter Billingsley[4], Sumana Chakravarty[4], Judith Epstein[1], Eileen Villasante[1], Thomas Richie[1], Barney Graham[2], Stephen Hoffman[4]

[1]Naval Medical Research Center, Silver Spring, MD, United States, [2]National Institutes of Health/VRC, Bethesda, MD, United States, [3]National Institutes of Health/National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, [4]Sanaria, Rockville, MD, United States

In animals, protective immunity induced by irradiated sporozoites (SPZ) is dependent on CD8+ T cells (mice, monkeys) and IFN-γ (mice). We therefore studied IFN-γ responses in 32 subjects immunized multiple times with escalating doses ($7.5x10^3$, $3.0x10^4$ or $1.35x10^5$ PfSPZ) of radiation-attenuated, purified, cryopreserved *Plasmodium falciparum* SPZ (PfSPZ Vaccine, Sanaria) by IV injection. There was a dose response in regard to protection. None of the volunteers were protected at the lowest total dosage and all were protected at the highest total dosage (R. Seder et al. submitted). This provided the opportunity to begin studying the association between immune responses and protection. Because the antigens involved in protective immunity induced by immunization with the PfSPZ Vaccine are unknown, we used IFN-γ ELISpot assays on freshly isolated PBMC to assess recall responses to pools of overlapping 15-mer peptides representing 5 pre-erythrocytic stage proteins, CSP, AMA1, SSP2/TRAP, LSA1 and CelTOS, comparing these responses to those recalled by PfSPZ or the blood stage antigen PfMSP1 as positive and negative controls, respectively. Responses to each of the 5 Pf antigens were of lower magnitude (25-75 sfc/10^6PBMC) than were responses to PfSPZ. Analysis is ongoing, but in general there was a dose response for PfSPZ and several antigens, most strikingly, AMA1 and SSP2/TRAP. The combination of small numbers and the dose responses make it difficult to assess whether ELISpot responses to any particular antigen were associated with protection, but there was an indication that responses to AMA1 and PfSSP2/TRAP may be so associated. Based on the premise that protective responses targeting multiple antigens could be additive, we summed the responses to the 5 tested antigens, and correlated this with responses to PfSPZ. The two magnitudes were similar, and positively correlated. These preliminary data provide a foundation for prospective studies designed to determine the targets and mechanisms of the high level protective immunity induced by PfSPZ.

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IMMUNIZING AGAINST MALARIA BY INDUCING ANTIBODY AND CD8 T CELL MEDIATED PROTECTION

Sumana Chakravarty[1], Minglin Li[2], Richard E. Stafford[2], Meredith L. Leong[3], Yun Wu[2], Edward E. Lemmens[3], Bill Hanson[3], Peter Lauer[3], Dirk G. Brockstedt[3], Thomas W. Dubensky, Jr.[3], Stephen L. Hoffman[4], B. Kim Lee Sim[4]

[1]Sanaria, Rockville, MD, United States, [2]Protein Potential, Rockville, MD, United States, [3]Aduro BioTech, Berkeley, CA, United States, [4]Protein Potential and Sanaria, Rockville, MD, United States

The malaria vaccine, RTS,S/AS01 is safe and delays the onset of clinical malaria by 30%-50% depending on age group. Protection is thought to be primarily mediated by antibodies against the repeat region and possibly CD4+ T cell responses against the C' terminus of the PfCSP. The vaccine does not induce meaningful CD8+ T cell responses. RTS,S/AS01 is not being considered for preventing malaria in non-immune travelers and elimination campaigns, because its protective efficacy is too low. A vaccine for these indications needs to provide >80% protective immunity for at least 6 months. We hypothesize that by adding highly functional, protective CD8+ T cell responses to antibody responses against the PfCSP, such protective immunity can be achieved. This response should be multifunctional as opposed to the high response obtained with adenovirus based vaccines that have not translated protective efficacy in humans. We are using live-attenuated Listeria monocytogenes (Lm) as a vaccine platform due to its demonstrated properties of effectively stimulating robust, multi-functional, cell-mediated immunity, as a result of its intracellular lifecycle and ability to directly infect, deliver antigen to, and stimulate DCs *in vivo*. We have developed an attenuated Lm-based vaccine platform (Lm ΔactAΔinlB) that has been evaluated in multiple clinical trials in patients with malignant and infectious diseases. This live-attenuated Lm ΔactAΔinlB strain is genetically defined with 2 virulence determinants deleted, resulting in a greater than 1,000-fold attenuation as compared to wild-type Lm, but retaining the immuno-stimulatory potency of the fully virulent wild-type pathogen. We used a prime-boost regimen combining selected molecular adjuvants formulated with recombinant PfCSP protein (rPfCSP) and Lm expressing PfCSP (Lm-PfCSP), to induce PfCSP-specific inhibitory antibodies and CD8+ and CD4+ T cell responses. We will discuss the high levels of inhibitory antibodies as assessed by inhibition of liver stage development and assess long term memory seen with our strategy.

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IMMUNE RESPONSES OF RHESUS MONKEYS TO A SELF-ASSEMBLING PROTEIN NANOPARTICLE (SAPN) VACCINE DISPLAYING *PLASMODIUM FALCIPARUM* CSP B- AND T-CELL EPITOPES

David E. Lanar[1], Margaret E. McCoy[1], Hannah E. Golden[1], Xiaoyan Zou[2], Zeyana S. Rivera[1], Qin Guo[1], Debleena Dasgupta[1], Stephen A. Kaba[1], David Fryauff[2], Walter R. Weiss[2], Randall F. Howard[3], Carter Darrick[3], Steven G. Reed[3], Vincent R. Gerbasi[2], Peter Burkhard[4]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Naval Medical Research Center, Silver Spring, MD, United States, [3]Infectious Disease Research Institute, Seattle, WA, United States, [4]University of Conneticut, Storrs, CT, United States

We have previously studied in mice the immune responses induced against *Plasmodium falciparum* circumsporozoite protein (PfCSP) epitopes using a self-assembling protein nanoparticle (SAPN) platform. As a path to testing this vaccine in humans, we conducted safety and immunogenicity studies in rhesus macaques. We hypothesized that an SAPN displaying B- and T-cell PfCSP epitopes would be safe and induce significant responses in macaques with and without the use of an adjuvant. Therefore, we constructed a PfCSP-KMY-SAPN displaying 60 copies of the PfCSP internal repeat sequence $(NANP)_4$ and 60 copies each of three previously identified human MHC-restricted CD8 T-cell epitopes (KPKDELDY, MPNDPNRNV and

YLNKQNSL). Monkeys received four immunizations with the PfCSP-KMY-SAPN with or without the adjuvant GLA-SE. No adverse events developed in any of the animals as a result of immunizations. Antisera and PBMC's were obtained and evaluated by multiple criteria to determine their PfCSP immune specificity. Titer to NANP repeats by ELISA was about $5x10^2$ following immunizations of PfCSP-KMY-SAPN in saline, but increased 20-fold to ~ $1x10^4$ in combination with GLA-SE. Passive transfer of purified IgG from rhesus immunized with PfCSP-KMY-SAPN/GLA-SE prevented infection of 100% of C57Bl/6 mice by a lethal challenge of a transgenic *P. berghei* sporozoite displaying full length *P. falciparum* CSP. Furthermore, serum from these same PfCSP-KMY-SAPN/GLA-SE-immunized monkeys inhibited *P. falciparum* sporozoite infection of primary human hepatocyte cultures by 90%. PBMC were purified and are undergoing evaluation for epitope-specific IFN-γ, IL-2 and TNFα responses. In conclusion, a PfCSP-KMY-SAPN vaccine for malaria was safe and immunogenic in rhesus monkeys. Immune responses to the vaccine were greatly enhanced if the nanoparticle was formulated with the adjuvant GLA-SE.

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PROFILING OF ANTIBODIES IN LYMPHOCYTE SUPERNATANTS (ALS) FROM *PLASMODIUM FALCIPARUM* INFECTED PATIENTS IN PERU

Li Liang[1], **Emmanuel Y. Dotsey**[1], Chris Hung[1], Katherine Torres[2], David Huw Davies[1], Peter D. Crompton[3], Joseph M. Vinetz[4], Philip L. Felgner[1]

[1]University of California Irvine, Irvine, CA, United States, [2]Malaria Laboratory, Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru, [3]Laboratory of Immunogenetics, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [4]University of California San Diego, San Diego, CA, United States

Currently available serologic assays cannot distinguish between circulating antibodies secreted by long-lived plasma cells generated in response to remote infections from antibodies secreted by plasmablasts generated in response to acute or recent infections. To address this technological gap in the context of malaria we developed a high throughput assay to profile Antibodies in Lymphocyte Supernatants (ALS) which are representative of antibodies secreted by circulating plasmablasts. Serum samples and peripheral blood lymphocytes were collected from Peruvian adults with either symptomatic or asymptomatic *Plasmodium falciparum* infection as well as from uninfected controls. Serum samples and supernatants from lymphocyte culture supernatants were probed against protein microarrays containing 500 *P. falciparum* and 500 *P. vivax* proteins. Strong antibody responses were detected in ALS from asymptomatic patients, whereas reactivity in symptomatic patients was much lower, and reactivity in control individuals was negligible. *P. falciparum* antigens differentially recognized by asymptomatic and symptomatic parasitemic individuals were identified. The antibody profiles of the corresponding serum samples were also determined and compared to ALS, and cross-reactivity with the *P. vivax* orthologous was also examined. These data demonstrate the feasibility of separately profiling the antigen specificity of antibodies from plasmablasts resulting from recent exposure, from antibodies circulating in serum that are derived from mature long lived plasma cells. Applied to various study designs involving natural and/or experimental infections with *P. falciparum* and other pathogens, this relatively simple technology will likely provide important insights into the nature of the antibody response to *P. falciparum* and other infections.

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DECIPHERING THE EXPRESSED ANTIBODY V GENE REPERTOIRE IN MALARIA

Severin Zinöcker[1], Jean-Nicolas Schickel[2], Eric R. Meffre[2], Susan K. Pierce[1]

[1]National Institutes of Health, Rockville, MD, United States, [2]Yale University, New Haven, CT, United States

Individuals living in malaria endemic areas gradually acquire conventional parasite-specific memory B cells (MBC) as well as a large population of atypical MBCs that are associated with chronic infectious diseases, including AIDS. At present, we know little about the molecular mechanisms underlying the generation of conventional and atypical MBCs in response to malaria. To gain insight into these processes we are sequencing the variable (V) gene segments of the immunoglobulin heavy (H) and light (L) chain genes from hundreds of conventional and atypical MBC clones from the peripheral blood of children and adults living in a malaria endemic area of Mali. The analyses of paired V_H and V_L sequences on the clonal level will allow us to determine the germline V_H and V_L gene usage in the conventional and atypical MBC population and the relationship between the two. In addition, the number and the nature of somatic hypermutations in V_H and V_L genes will provide insights to the role of antigen-driven selection in the development of those cell types. By this analysis we aim to gain a better understanding of the generation of both conventional and atypical MBC during the acquisition of antibody-dependent immunity in malaria.

1442

THE PFRH AND EBA INVASION LIGANDS OF *PLASMODIUM FALCIPARUM* ARE IMPORTANT TARGETS OF HUMAN INHIBITORY ANTIBODIES AND FUNCTION TO EVADE NATURALLY ACQUIRED IMMUNITY

Linda Reiling[1], Kristina E. Persson[2], Freya J. Fowkes[1], Fiona J. McCallum[3], Nimmo Gicheru[4], Jack S. Richards[1], Christine Langer[1], Alan F. Cowman[5], Peter M. Siba[6], Chetan Chitnis[7], Takafumi Tsuboi[8], Ivo Mueller[5], Kevin Marsh[4], **James G. Beeson**[1]

[1]The Burnet Institute, Melbourne, Australia, [2]Karolinska Institutet, Stockholm, Sweden, [3]Army Malaria Institute, Enoggera, Australia, [4]Kenya Medical Research Institute, Kilifi, Kenya, [5]The Walter and Eliza Hall Institute, Melbourne, Australia, [6]Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea, [7]International Centre for Genetic Engineering and Biotechnology, New Delhi, India, [8]Ehime University Graduate School of Medicine, Toon, Japan

Acquired antibodies are important in human immunity to malaria and can inhibit *Plasmodium falciparum* invasion of erythrocytes, but key target antigens of protective and functional antibodies are largely unknown. Phenotypic variation by *P. falciparum* merozoites can mediate the evasion of inhibitory antibodies, contributing to the capacity of *P. falciparum* to cause repeated and chronic infections. However, antigens involved in mediating immune evasion have not been defined, and studies of the function of human antibodies are limited. We have studied immune responses to *P. falciparum* reticulocyte binding homologues (PfRh1, PfRh2, PfRh4, and PfRh5) and erythrocyte-binding antigens (EBA175, EBA140, and EBA181), which are two families of invasion ligands that play important roles in invasion of erythrocytes and are potential vaccine candidates. We used novel and complementary approaches to determine the importance of PfRh proteins and EBAs as targets of protective human and invasion-inhibitory antibodies, and we defined their role in contributing to immune evasion through variation in function. We evaluated the invasion-inhibitory activity of acquired antibodies from malaria-exposed children and adults using *P. falciparum* lines with targeted disruption of genes encoding different PfRh and EBA ligands in functional assays, and the invasion-inhibitory activity of human affinity-purified antibodies to PfRh and EBA ligands. Furthermore, we examined the association between antibodies to different PfRh and EBA ligands

and protection from malaria in a longitudinal cohort study of children. Considering all data together, our findings provide important evidence that PfRh and EBA ligands are major targets of invasion-inhibitory and protective human antibodies, and that variation in the expression and function of the PfRh and EBAs mediates evasion of acquired antibodies. This knowledge will help to advance malaria vaccine development and understand how the immune response targets multiple invasion ligands to overcome the capacity of *P. falciparum* for immune evasion.

1443

DEMONSTRATION OF ENHANCED STRAIN-SPECIFIC *PLASMODIUM FALCIPARUM* MULTIFUNCTIONAL T CELL CYTOKINE EXPRESSION AMONG MALIAN CHILDREN IMMUNIZED WITH THE FMP2.1/AS02A VACCINE

Shawna F. Graves[1], Bourema Kouriba[2], Issa Diarra[2], Amadou Niangaly[2], Modibo Daou[2], Drissa Coulibaly[2], Yamoussa Keita[2], Matthew B. Laurens[3], Johan Vekemans[4], W. Ripley Ballou[4], David Lanar[5], Sheetij Dutta[5], D. Grey Heppner[5], Lorraine Soisson[6], Carter L. Diggs[6], Mahamadou A. Thera[2], Ogobara K. Doumbo[2], Christopher V. Plowe[3], Marcelo B. Sztein[1], Kirsten E. Lyke[1]

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

Based on *Plasmodium falciparum* (Pf) apical membrane antigen 1 (AMA1) from strain 3D7, the malaria vaccine candidate FMP2.1/AS02A was tested in a Phase 2 clinical trial in 400 Malian children (aged 1-6 years) randomly assigned to receive 3 doses of the AMA1 vaccine or a control rabies vaccine on days 0, 30 and 60. A subset of 10 Pf(-) (i.e., no clinical Pf episodes) and 12 Pf(+) (clinical malaria episodes with parasites with 3D7 or Fab9-type AMA1 cluster 1 loop [c1L]) AMA1 recipients, and 10 controls were randomly chosen for analysis. Peripheral blood mononuclear cells (PBMCs) isolated on days 0, 90 and 150 were stimulated with full-length 3D7 AMA1 and with c1L from strains 3D7 and Fab9 (identical to 3D7 c1L except for amino acid 197) to assess allele-specific cell-mediated responses. T cell expression of INF-γ, TNF-α, IL-2, and/or IL-17A was analyzed by 11-color flow cytometry. Among AMA1 recipients, 19/21 evaluable samples stimulated with AMA1 demonstrated significantly increased levels of IFN-γ, TNF-α and IL-2 derived from CD4+ T cells by D150 compared to 0/10 in the control group (P<0.0001). CD4+ cells expressing both TNF-α and IL-2 were increased in Pf(-) children (median=28.4% of cytokine-expressing cells) compared to Pf(+) children in the AMA1 vaccine group (median=8.6% of cytokine-expressing cells). Low prevalence of double- (IFN-γ+TNF-α+) and triple-positive (IFN-γ+TNF-α+IL-2+) CD4+ cytokine-expressing cells were noted. When PBMCs were stimulated with c1L from 3D7 and Fab9 separately, 5/19 AMA1 recipients with an AMA1-specific CD4+ response had a significant response to one or both c1L. This suggests that AMA1 vaccination induced an AMA1-specific CD4+ response; however, recognition of the vaccine antigen is not dependent upon c1L alone. In summary, AMA1-specific T cell cytokine expression was notably increased in children vaccinated with an AMA1-based vaccine compared to rabies. The possible role of CD4+ TNF-α+IL-2+-expressing T cells in vaccine-induced strain-specific protection against clinical malaria requires further exploration.

1444

DETECTION AND SEMIQUANTITATION OF VENOM AND ANTIVENOM IN THE BLOOD OF TWENTY PATIENTS WITH EVIDENT NEUROTOXIC ENVENOMATION IN GUINEA

Blanca Ramos-Cerrillo[1], Mamadou C. Baldé[2], Achille Massougbodji[3], **Jean-Philippe Chippaux**[4], Roberto P. Stock[1]

[1]Instituto de Biotecnologia (Universidad Nacional Autonoma de Mexico), Cuernavaca, Mexico, [2]Institut Pasteur de Guinée, Kindia, Guinea, [3]Faculté des Sciences de la Santé (UAC), Cotonou, Benin, [4]Institut de Recherche pour le Développement, Paris, France

In Guinea, elapids are responsible for about 20% of envenomations. Recent studies have shown that case fatality rate falls between 15 and 30% regardless of treatment. We obtained blood samples from 20 patients who presented typical neurotoxic syndromes. All patients were treated with 40 ml of antivenom neutralizing the main species of Elapidae in the region: *Dendroaspis polylepis* (Dp), *D. viridis* (Dv), *Naja melanoleuca* (Nm) and *N. nigricollis* (Nn). Blood samples were spotted onto Guthrie paper before antivenom treatment (hour 0, H0) and two hours after antivenom administration (H2). The samples were analyzed by a custom sandwich ELISA for venom of each of the four species using rabbit antibodies purified against the venom of each species by affinity chromatography and adsorbed against the other three species. The presence of antivenom was also established for all samples. Five patients died. Samples on H0 were missing for 2 patients and samples on H2 were missing for another 2. Of the 18 H0 samples tested, a clear and high venom signal was detected in 4: 2 patients who died were strongly positive for Dp, including a patient who died before treatment, 1 patient was strongly positive for Dv (who also died) and one patient who survived was strongly positive for Nn. Antivenom was detected in the H2 samples of 14 patients. For the 2 patients for whom a clear venom signal was detected at H0 and for whom H2 samples were available, residual venom was detected using an ELISA assay based on immunopurified horse antibodies. A competition ELISA test in which venom was titrated with increasing amounts of antivenom showed that the venom detected by means of this ELISA assay is likely to be free residual venom. The ELISA assays performed on samples spotted and dried on Guthrie paper are robust, very specific but not very sensitive. They have nonetheless permitted, for the first time, an immunodiagnosis of the species of African elapid causing envenomation in 4 of 18 patients, including those 3 (of 5) who died during the study. They have also shown that residual "free" venom was present in two patients after antivenom administration, suggesting that the dose of antivenom administered may have been insufficient to completely neutralize the venom present in some of these victims of snakebite.

1445

USE OF JAPANESE ENCEPHALITIS VACCINE IN U.S. TRAVEL MEDICINE PRACTICES IN GLOBAL TRAVEPINET

Bhushan Deshpande[1], Sowmya R. Rao[2], Gary Brunette[3], Mark J. Sotir[3], Mark D. Gershman[3], Emily S. Jentes[3], Susan L. Hills[4], Marc A. Fischer[4], Edward T. Ryan[5], **Regina C. LaRocque**[5]

[1]Tufts University, Medford, MA, United States, [2]University of Massachusetts Medical School, Worcester, MA, United States, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States, [4]Centers for Disease Control and Prevention, Fort Collins, CO, United States, [5]Massachusetts General Hospital, Boston, MA, United States

Japanese encephalitis (JE) vaccine is recommended for high-risk travelers to Asia and the western Pacific. Few data regarding the use of this vaccine in clinical practice are available. We evaluated international travelers to JE-endemic regions who were seen at U.S. Global TravEpiNet (GTEN) sites between September 2009 and August 2012, when IXIARO was in use. We categorized travelers as higher or lower risk for JE based on the destination country, travel plans, and duration of travel. We compared the demographic and clinical features of higher and lower JE risk travelers and

performed multivariable analyses to identify factors that were associated with travelers not being offered or declining the JE vaccine. We identified 711 higher JE risk travelers and 7,578 lower JE risk travelers in our analysis. Higher JE risk travelers were younger than lower JE risk travelers (median age 29 years vs. 40 years, p<0.001) and traveled for longer durations of time (median 50 days vs. 14 days, p<0.001). 43% of higher JE risk travelers were offered the JE vaccine, and 62% of these travelers accepted it. Short time to departure, rural travel, travel to visit friends and relatives, leisure travel, and travel for humanitarian service work were each independent predictors of declining the JE vaccine. Additionally, 40% of higher JE risk travelers were judged by their clinician to not require the JE vaccine. Travel to visit friends and relatives, leisure travel, travel to India, and travel to China were independent predictors of a higher JE risk traveler not being offered the JE vaccine. Clinicians did not recommend the JE vaccine to many travelers who met the indications offered by the Advisory Committee on Immunization Practices, and there are disparities with regard to subpopulations that receive the vaccine.

1446

EOSINOPHILIA AS A POTENTIAL SURROGATE FOR THE DIAGNOSIS OF STRONGYLOIDIASIS IN AN IMMIGRANT POPULATION AND THE UTILITY OF ABSENT SS-NIE ANTIBODIES AS A BIOMARKER FOR CURE

Rojelio Mejia[1], Kathryn E. Spates[2], Nicole Holland[2], Amara G. Pabon[2], JeanAnne Ware[2], Thomas B. Nutman[2]

[1]National School of Tropical Medicine, Baylor College of Medicine, Houston, TX, United States, [2]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

Determining the cause of persistent eosinophilia in immigrants to the United States can be hampered by costs needed to evaluate suspected parasitic infections. Thus, diagnosing eosinophilia-causing helminth infections by stool examination or serology is often beyond the means of community health clinics that commonly serve immigrant populations. To define the causes of persistent eosinophilia among an immigrant population seen at a single community free health clinic, 54 patients (originally from Central and South America, Africa, Asia and the Middle East) who arrived in the United States 1-27 years (median 7 years) previously--were found to have an absolute eosinophil count (AEC) >500/uL and were referred to the National Institutes of Health for further testing. Of the 54 referred patients, 43 (80%) had positive *Strongyloides stercoralis* (Ss)-specific serology. 1/43 (2%) also had schistosomiasis, 3/43 (7%) hookworm, and 2/43(5%) trichuriasis. There were no differences in baseline eosinophil counts and serum IgE levels between those with Ss and the 11/54 without (probably reflecting the referral criteria). All patients with a definitive parasitologic diagnosis received ivermectin and (when appropriate praziquantel and/or albendazole) treatment and followed over the course of a year. Not unexpectedly, there was a dramatic and significant (p<0.0002) decrease in AEC following treatment with all returning to normal levels by 1 year. IgE levels also fell dramatically following treatment. Most importantly, antibodies to the *Strongyloides*-specific recombinant antigen (Ss-NIE) using a luciferase immunoprecipitation assay (LIPS) also became negative in all those with Ss treated successfully with ivermectin. Thus, in community clinics that provide health care to immigrants well after arrival in the United States, an AEC can be used as a surrogate for stool examination, and serology may be a trigger for empiric treatment when testing is limited by cost. If available, newer serologic tests may replace insensitive stool examinations as tests of cure.

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RISK FACTORS AND SEROPREVALENCE OF *TRYPANOSOMA CRUZI* INFECTION IN TEXAS

Melissa S. Nolan, Peter Hotez, Laila Woc-Colburn, Kristy Murray

Baylor College of Medicine, Houston, TX, United States

Chagas' disease has emerged as an important neglected tropical disease in the United States; particularly in Texas. Chagas' disease is caused when the parasite *Trypanosoma cruzi* (*T. cruzi*) is transmitted to humans by a *Triatominae* insect. One-third will develop chronic infection that can result in cardiac myopathy and death. This study aimed to determine risk factors and estimate disease burden of Chagas disease in Texas. Data was collected from five major blood centers in Texas on those tested for *T. cruzi* from 2008-2012. We only included original donations tested from each donor, and duplicate donations were excluded for seroprevalence analysis by stratification. Risk factors were analyzed by zip-codes with and without a reported case to the Chagas' Biovigilance Network and/or a major Texas blood center. We found 1 per 3,500 population were positive for *T. cruzi* in Texas. Seroprevalence was similar between genders. Infection rate increased with age with ages 41-50 (40 per 100,000) and 51+ (41 per 100,000) having the highest infection rate. As expected, Hispanics had the highest infection rate (43 per 100,000). Caucasians (14 per 100,000) and African Americans (24 per 100,000) had the lowest infection rates. We calculated a total cost to society of $215 million for these cases. *T cruzi* positive cases were significantly more likely to live in zip-codes that have a higher percentage of foreign born residents (p<0.001) and urban land use (p<0.001). In conclusion, blood Centers are an important component in understanding *T. cruzi* transmission in Texas. Approximately 1 per 3,500 blood donors test positive for *T. cruzi*. Chronic cases accrue $215 million in lifetime societal cost to Texans. Minorities, urban areas, and areas with high foreign born population are at highest risk for *T. cruzi* infection.

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SPATIAL DISTRIBUTION OF PODOCONIOSIS IN RELATION TO ENVIRONMENTAL FACTORS IN ETHIOPIA: A HISTORICAL REVIEW

Kebede Deribe[1], Simon J. Brooker[2], Rachel L. Pullan[3], Asrat Hailu[4], Fikre Enquselassie[5], Richard Reithinger[6], Melanie Newport[7], Gail Davey[7]

[1]School of Public Health, Addis Ababa University, Addis Ababa, Ethiopia and Brighton and Sussex Medical School, Falmer, Brighton, United Kingdom, Addis Ababa, Ethiopia, [2]Kenya Medical Research Institute-Wellcome Trust Research Programme, Nairobi, Kenya and Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, United Kingdom, [4]School of Medicine, Addis Ababa University, Addis Ababa, Ethiopia, [5]School of Public Health, Addis Ababa University, Addis Ababa, Ethiopia, [6]Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, United Kingdom, School of Public Health and Health Sciences, George Washington University, Washington, DC, RTI International, Washington, DC, United States, [7]Brighton and Sussex Medical School, Falmer, Brighton, United Kingdom

An up-to-date and reliable map of podoconiosis is needed to design geographically targeted and cost-effective intervention in Ethiopia. Identifying the ecological correlates of the distribution of podoconiosis is the first step for risk and distribution maps. The objective of this study was to investigate the spatial distribution and ecological correlates of podoconiosis using historical and contemporary survey data. Data on the observed prevalence of podoconiosis were abstracted from published and unpublished literature into a standardized database, according to strict inclusion and exclusion criteria. In total, 10 studies conducted between 1969 and 2012 were included through structured searches, and data were available for 401,674 individuals older than 15 years of age from 229 locations. A range of high resolution environmental factors were

investigated to determine their association with podoconiosis prevalence, using logistic regression. The prevalence of podoconiosis in Ethiopia was estimated at 3.4% (95% CI: 3.3%-3.4%) with significant regional variation. We identified significant associations between altitude, mean annual Land Surface Temperature (LST), mean annual precipitation, topography of the land and fine soil texture and high prevalence of podoconiosis ($p<0.001$). The derived maps indicate both widespread occurrence of podoconiosis and a marked variability in prevalence of podoconiosis, with prevalence typically highest at altitudes >1500 m above sea level (masl), with >1500 mm annual rainfall and mean annual LST of 19-21°C. No (or very little) podoconiosis occurred at altitudes <1225 masl, with annual rainfall <900 mm, and mean annual LST of >24°C. Podoconiosis remains a public health problem in Ethiopia over considerable areas of the country, but exhibits marked geographical variation associated in part with key environmental factors. This is work in progress and the results presented here will be refined in future work.

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DISCOVERING THE PATHOGENS OF CENTRAL NERVOUS SYSTEM INFECTION IN NEPAL

Abhisek Giri[1], Amit Arjyal[1], Samir Koirala[1], Abhilasha Karkey[1], Sabina Dangol[1], Sudeep Dhoj Thapa[1], Olita Shilpakar[1], Rishav Shrestha[1], **Radheshyam Krishna KC**[2], Le Van Tan[3], Jeremy Farrar[4], Buddha Basnyat[1]

[1]Oxford University Clinical Research Unit-Patan Hospital, Kathmandu, Nepal, [2]Patan Academy of Health Sciences, Kathmandu, Nepal, [3]Oxford University Clinical Research Unit-Vietnam, Ho Chi Minh City, Vietnam, [4]Center for Tropical Medicine, Oxford University, Oxford, London, United Kingdom

Central nervous system (CNS) infection is one of the common causes of hospital admission in Nepal. Due to the absence of specific tests to diagnose the definitive cause of meningitis, the treatment is often empirical. The condition is more challenging when there is prior use of antibiotics. Such-conditions alter the possible outcomes, which ultimately affects treatment and management. Therefore, the aim of this study is to find the possible etiological agents responsible for meningitis in adults in Nepal. We conducted a prospective hospital based study to identify the possible pathogens of CNS infections in adults admitted in Patan Hospital from February 2009-April 2011. The pathogens of CNS infections were confirmed in cerebrospinal fluid (CSF) using molecular diagnostics, culture (bacteria) and serology. 87 patients were recruited for the study and the etiological diagnosis was established in 38% (n=33). The bacterial pathogens identified were Neisseria meningitidis (n=6); Streptococcus pneumoniae (n=5) and *Staphylococcus aureus* (n=2) in 13/87(14%). Enteroviruses were found in 12/87 (13%); Herpes Simplex virus (HSV) in 2/87(2%). IgM against Japanese encephalitis virus (JEV) was detected in CSF of 11/73 (15%) tested samples. In conclusion, our study is the first (RT) PCR and serology based CSF analysis from Kathmandu, Nepal that attempts to indentify the causative organisms of infectious syndromes of the central nervous system in adults. JEV and enteroviruses were the most commonly detected pathogens.

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SEVERE MALARIAL ANEMIA IS ASSOCIATED WITH LONG-TERM NEUROCOGNITIVE IMPAIRMENT

Paul Bangirana[1], Robert O. Opoka[1], Richard Idro[1], James S. Hodges[2], Michael J. Boivin[3], Chandy C. John[2]

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

Cerebral malaria (CM) is associated with long-term cognitive impairment in children 5 years of age and older. No prospective studies have assessed cognitive impairment in children with CM <5 years of age, or in children with severe malarial anemia (SMA), a more common manifestation of severe malaria that is estimated to affect > 5 million children annually. Children <5 years of age who presented to Mulago Hospital, Kampala, Uganda, with CM (n=80) or SMA (n=86) were assessed for overall cognitive function, attention, and declarative memory one week after discharge and 6 and 12 months later. Age-adjusted z-scores for each domain were generated from the scores of 61 healthy community children (CC), who were also tested at enrollment and 6 and 12 months later. Groups were compared using mixed linear models. For the full one-year period of follow-up, children with CM had significantly worse scores than CC in overall cognitive function (-1.00 vs -0.12; $P<0.0001$), attention (-0.51 vs -0.06; $P=0.004$), and declarative memory (-0.41 vs 0.04; $P=0.0003$). Children with SMA also had significantly worse scores than CC in overall cognitive function (-0.52 vs -0.12; $P<0.0001$) and attention (-0.25 vs -0.06; $P=0.03$), but not declarative memory (-0.03 vs 0.04; $P=0.99$). Scores for overall cognitive function and attention did not differ significantly between children with CM vs. SMA. In children <5 years of age, CM is associated with long-term impairment in overall cognitive function, attention, and declarative memory, and SMA is associated with long-term impairment in overall cognitive function and attention. SMA may be a major cause of long-term neurocognitive impairment in children in sub-Saharan Africa.

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EPIDEMIOLOGICAL, CLINICAL AND LABORATORY DESCRIPTION OF ONCHOCERCIASIS IN AN AREA OF HIGH PREVALENCE - JIMMA, ETHIOPIA, 2013

Francisca Abanyie[1], Paul Cantey[1], Sindew Mekasha[2], Markos Sleshi[2], Nicholas Ayebazibwe[3], Shoshana Oberstein[1], Elizabeth Thiele[1], Anne Moore[1], Mark Eberhard[1], Vitaliano Cama[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia, [3]African Field Epidemiology Network, Kampala, Uganda

Onchocerciasis, transmitted by blackflies, still infects at least 37 million people worldwide, but elimination of onchocerciasis through mass drug administration (MDA) with ivermectin is feasible in parts of Africa. However, evidence-based tools to evaluate program endpoints are lacking. Clinical specimens characterized with epidemiologic, clinical, and laboratory data were collected and analyzed to evaluate the existing diagnostic tests for onchocerciasis and to identify the best tests to measure programmatic endpoints. Five-hundred specimens were collected in three onchocerciasis-endemic areas in Jimma, Ethiopia, where one round of ivermectin MDA had been given five months before the study. Laboratory analysis in country included blood smears to detect *Loa loa* and *Mansonella perstans*, immunochromatographic card tests (ICT) for filariasis, and skin snip examination for *Onchocerca volvulus*. Plasma, serum, blood smears, dried blood spots, and preserved skin snips were sent to CDC for further analysis. The median age of participants was 45 (range 6-90 years); 276 (55%) were male. Though only 57 (11%) participants reported living near a river, 244 (49%) spent the majority of the day near rivers where blackflies typically bite. Eight (2%) participants had *O. volvulus* microfilaria present in the anterior chamber (N=2) or cornea (N=6). At least one skin nodule was noted in 319 (64%) participants (range 1-11); 312 (62%) had other onchocercal skin manifestations; 74 (15%) had evidence of lymphedema. The skin snip was positive for *O. volvulus* in19 (4%) participants, with a mean load (average of both snips) among those with at least one positive snip of 19.5 microfilaria/slide (range 0.5-180); 15 (3%) had positive ICTs. The paucity of positive skin snips despite the high prevalence of nodules is unexplained and needs further investigation. *Wuchereria bancrofti* may be co-endemic in the areas studied. Additional laboratory evaluation is pending. The performance of these tests in the African context will help determine their use in the evaluation of elimination program endpoints.

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COMPLEMENTARY USE OF COMPREHENSIVE SURVEILLANCE AND TRANSMISSION ASSESSMENT SURVEYS FOR ASSESSING PERSISTENCE OF LYMPHATIC FILARIASIS IN SRI LANKA FOLLOWING MASS DRUG ADMINISTRATION

Udaya S. Ranasinghe[1], Ramakrishna U. Rao[2], Kumara C. Nagodavithana[1], Sandhya D. Samarasekera[1], Sunil Settinayake[1], Gary J. Weil[2]

[1]Antifilariasis Campaign, Ministry of Health and Nutrition, Colombo, Sri Lanka, [2]Washington University School of Medicine, St. Louis, MO, United States

The Sri Lankan Anti-Filariasis Campaign (AFC) provided mass drug administration (annual diethylcarbamazine plus albendazole) according to WHO guidelines to some 10 million people in 8 endemic districts between 2002 and 2006. All districts met WHO criteria for lymphatic filariasis (LF) elimination in 2008, but spot surveys showed low-level persistence of microfilaremia (Mf) in some sentinel sites. Comprehensive surveillance of suspected hotspots (in 2 public health inspector areas per district) was initiated in early 2010, and WHO recommended TAS surveys were conducted in 2012-13. Comprehensive surveillance included community surveys for Mf and filarial antigenemia (ICT), school surveys for ICT and anti-filarial antibodies (Bm14 ELISA) in children 6-8 years of age, and mosquito surveys to detect filarial DNA in *Culex* mosquitoes collected by gravid traps (molecular xenomonitoring, MX). TAS surveys involved ICT testing of ~1,500 children in 30 to 35 randomly selected schools in each evaluation unit. Provisional targets for LF elimination in hotspot surveys were <0.5% for Mf (community surveys), <2% for ICT (community), <2% for antibody in first grade primary school children, and <0.25% for filarial DNA in mosquitoes. We now report results from 13 hot spot surveys that were conducted in 6 formerly endemic districts. Community Mf and ICT prevalence rates were between 0-0.9% and 0-3.4%, respectively. ICT rates in school children were < 1% in all 13 sites, but antibody rates in school children exceeded 2% in 9 sites. Filarial DNA rates in mosquitoes exceeded the target rate in 7 of 13 study sites, and all of these LF indicators exceeded our targets in one site. Thus, many hot spots had evidence of low level persistence of LF some 6 years after MDA. TAS survey results showed that ICT rates in primary school children satisfied WHO targets in all 8 districts. These results suggest that antibody testing of children and MX are more sensitive tools for detecting low-level persistence of filariasis in communities than TAS. We recommend using enhanced surveillance tools to complement TAS surveys for post-MDA surveillance. We also recommend close follow-up for areas that failed to meet elimination targets to determine whether further intervention is required in these areas.

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ACTIVE TRANSMISSION OF FILARIASIS IN ZANZIBAR AFTER MDA HAD BEEN STOPPED FOR FIVE YEARS

Maria Rebollo Polo[1], Khlafan Mohammed[2], Said Mohammed[3], Ali Iddi Simba Khamis[2], Brent Thomas[1], Jorge Cano[4], Lorenzo Savioli[5], Moses J. Bockarie[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]MoH, Zanzibar, United Republic of Tanzania, [3]Public Health Laboratory, Chake Chake, Pemba, Zanzibar, United Republic of Tanzania, [4]London School of Hygiene Tropical Medicine, London, United Kingdom, [5]World Health Organization, Geneva, Switzerland

The Global Programme to Eliminate Lymphatic Filariasis (GPELF) recommends annual mass drug administration for 4-6 years to interrupt transmission of the disease. Zanzibar, in the United Republic of Tanzania, was the first country to complete five rounds of treatment using a combination of albendazole and ivermectin at 100% geographic coverage and achieving effective coverage rate of over 65% during all five years. MDA implemented through filarial prevention assistants (FPAs) selected that were resident in the communities and aware of public health activities to various degrees. Total treatment coverage averaged from 70 to 80% in all five rounds mainly due to a very effective social mobilization programme. Impact assessment at two sentinel sites showed that the prevalence and intensity of microfilaria decreased significantly after the first round of MDA and the decline continued after subsequent MDA rounds. MDA was stopped in 2006 after sentinel site surveys revealed prevalence below 1%.In early 2012, transmission assessment surveys (TAS) were conducted to determine if transmission of LF had been interrupted on the two islands. The TAS surveys involved a total of 72 schools; 36 from each of the two Evaluation Units (EUs) in Pemba and Unguja islands. A total of 1298 children were surveyed on Pemba where 70 (5.4%) were found to be positive. In Unguja, 19 (0.95%) of the 1980 pupils tested were positive. The EU on Pemba exceeded the critical cut-off of 18, thereby failing the TAS criteria and implying that transmission had resumed. On the other hand the number of positives from Unguja just fell short of 20, suggesting that the level of exposure, though high, may not be sufficient to sustain transmission. Based on the TAS results it was recommended that MDA be restarted on both islands in 2013 because of the efficiency of the *Culex* vector. Another TAS will be conducted after two rounds. This study confirms the recommendation that effective surveillance and possible continued actions after the achievement of the elimination target may be required to prevent re-establishment of transmission

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TRANSMISSION OF ONCHOCERCIASIS IN CENTRAL NIGERIA: ONGOING TRANSMISSION OR DISEASE ELIMINATION?

Darin Evans[1], Kal Alphonsus[2], John Umaru[2], Abel Eigege[2], Ellias Pede[3], Christopher Umbugadu[4], Carlos Gonzales[5], Frank Richards[1]

[1]The Carter Center, Atlanta, GA, United States, [2]The Carter Center, Jos, Nigeria, [3]Plateau State Ministry of Health, Jos, Nigeria, [4]Nasarawa State Ministry of Health, Lafia, Nigeria, [5]CCI, Chula Vista, CA, United States

Mass drug administration (MDA) with ivermectin is the WHO recommended strategy for control of onchocerciasis. Recent evidence has shown that after 15-17 years of treatment, elimination of the disease in Africa may be possible. In Plateau and Nasarawa states in North-Central Nigeria, MDA has been ongoing since 1991. Since 2000, albendazole has been co-administered with ivermectin for treatment of lymphatic filariasis (LF), which is co-endemic. In 2009, 5 districts were determined to have stopped LF transmission. We set out to evaluate the status of onchocerciasis transmission in these 5 districts to determine onchocerciasis transmission had also been interrupted. Using the 2001, WHO criteria for elimination of onchocerciasis, we sought to achieve a microfilariae (MF) and seroprevalence of <1/1,000 infected individuals and a rate of infective blackflies of <0.05/1,000. We evaluated adults and children in six sentinel sites and children only in eight spot-check villages. Skin snips, blood spots, and nodules were collected and fly catches were conducted at six river sites. We sampled a total of 5,182 persons: 4,441 children ages 3 to 12 and 746 adults ≥20 years in 14 communities. In adults, Mf prevalence had decreased 99.3% from a mean baseline of 43.0% to 0.27% (p<0.001). In children, no Mf were detected but a seroprevalence of 0.16% (n=7, 0.32% upper 95%CI) was found. A total of 1,568 blackflies were assessed in six capture sites. While no infective larvae were found, the number of flies caught was insufficient for determining whether transmission has been interrupted. In conclusion, current criteria from APOC use parasitologic and entomologic indicators for determining elimination status. In areas where the blackfly vector is less abundant, however, the number of flies needed to definitively decide may not be possible to obtain in a timely manner. In Plateau and Nasarawa states, we have found that, while we meet the parasitologic criteria, we cannot achieve the necessary number of flies needed to definitively determine if transmission has been interrupted despite the fact that no infective flies have been found. In this case, we have opted to include the 2001 WHO criteria to examine serologic evidence in children and have found that transmission may be ongoing.

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MALARIA AND FILARIASIS COINFECTIONS IN PAPUA NEW GUINEA

Daniel J. Tisch[1], Rajeev K. Mehlotra[1], Zachary Kloos[1], Peter M. Siba[2], James W. Kazura[1], Peter A. Zimmerman[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]Papua New Guinea Institute for Medical Resarch, Goroka, Papua New Guinea

Malaria and lymphatic filariasis (LF) elimination programs are predicated on efficient and effective surveillance and monitoring tools. Simultaneous detection of all four primary malaria species and *Wuchereria bancrofti* (Wb) using a post-PCR oligonucleotide ligation detection reaction-florescent microsphere assay (LDR-FMA) has been recently demonstrated in samples from the Dreikikir region of Papua New Guinea where malaria and filariasis are co-endemic and transmitted by Anopheline mosquitoes. In this setting mass drug administration has been deployed against LF beginning in the mid 1990s and long-lasting insecticide-treated nets were distributed in 2009. The present study evaluates a population of 2700 individuals in this region to quantify co-infection dynamics and characterize the complex epidemiology of these important parasitic diseases. Overall, our results showed that 84.1% of the individuals tested were assay-positive for at least one malaria species and 13.4% were positive for Wb. Among individuals infected with *Plasmodium* species parasites, 38.0%, 29.4%, 13.4%, and 3.3% of individuals were infected with 1, 2, 3, or 4 species of malaria, respectively. Diagnosis of Wb infection prevalence did not differ significantly according to the quantity of malaria species co-infections (p=0.350), even after stratifying for intensity of Wb infection (using ICT card grade or microfilaremia) or malaria LDR-FMA optical densities. Furthermore, Wb prevalence was not significantly different among malaria positive or negative individuals (13.6% vs 12.0%, p = 0.380). Interestingly, we observed that Wb infections were slightly more common in malaria infected (37.6) vs. uninfected individuals (28.0%) (p=0.044) among the subset of individuals residing in a geographic area traditionally characterized as having higher LF transmission prevalence. 35.6% of individuals in the higher LF transmission site and 7.6% of individuals in the lower LF transmission site were Wb positive by LDR-FMA whereas malaria prevalence was similar across sites (79.5% and 85.6%, respectively). Simultaneous multiple parasite detection such as LDR-FMA may be useful to integrated disease monitoring and elimination strategies.

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EVALUATION OF TEN YEARS IMPACT OF IVERMECTIN TREATMENT FOR ONCHOCERCIASIS ON LYMPHATIC FILARIAIS: A CASE STUDY FOR THREE OVERLAPPING DISTRICTS IN TANGA REGION, TANZANIA

Upendo J. Mwingira[1], **Maria J. Chikawe**[1], Mwelecele Malecela[2], Oscar Kaitaba[3], Rehema Maggid[4], Aferwork Hailemariam[5], Andreas Nshala[3]

[1]NIMR/NTD Control Programme, Dar es Salaam, United Republic of Tanzania, [2]NIMR, Dar es Salaam, United Republic of Tanzania, [3]NTD Control Programme, Dar es Salaam, United Republic of Tanzania, [4]NTD Control Programme, Tanga, United Republic of Tanzania, [5]APOC, Ougadougou, Burkina Faso

Tanzania is endemic with 5 of the PCT targeted NTDs namely Lymphatic Filariasis - LF, Onchocerciasis, Trachoma, Schistosomiasis and Soil Transmitted Helminthiasis - STH. MDA activities implemented in phases in various implementation units. Tanga region has been implementing consecutive Ivermectin MDA for Onchocerciasis control since 2000 and Ivermectin + Albendazole MDA for LF since 2004 and has completed over 5 effective Mass Drug Distribution rounds and the average coverage being above 65%. The study aim was to evaluate the impact of 10 rounds of Ivermectin treatment for Onchocerciasis on Lymphatic Filariais. The Survey districts were Lushoto, Muheza and Korogwe. Six sites for lymphatic filariasis were selected from the participating districts. One village with high prevalence of Onchocerciasis and another one with high prevalence of Lymphatic Filariasis were selected from each district. All hamlets in selected villages were surveyed. A cluster survey was applied involving communities. For LF survey, eligible population were individuals aged 5 years and above. A systematic random sampling of households was done to get 600 participants from each village. Enrolled individuals from the community were tested for Circulating Filarial Antigen (CFA) using ICTs. 100microlitres of blood sample from participants was collected from a finger prick, ICT was done and results provided after 10 minutes. For all ICT positive results night blood was collected between 10pm and midnight and microfilaria(mf) count was done using counting chamber technique. A total of 1887 people participated in the LF survey, 1020 (54.1%) were females and 867(45.9%) were males. All of the participants were tested for CFA with ICT, 40(2.1%) were ICT positive. Mf count was done to 34 of the ICT positives and 6 were positive. Results indicate that LF is still prevalent in the evaluated districts with different CFA prevalence levels and thus MDA should continue for few more round before conducting Transmission assessments survey (TAS).

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FORECASTING DEMAND FOR ONCHOCERCIASIS TREATMENT TO ACHIEVE ELIMINATION AND ERADICATION

Young Eun Kim[1], Jan H.F. Remme[2], Peter Steinmann[1], Fabrizio Tediosi[1]

[1]Swiss Tropical and Public Health Institute, Basel, Switzerland, [2]Independent Consultant, Ornex, France

Recent evidence indicates that mass onchocerciasis treatment with ivermectin can interrupt transmission and eliminate the parasite in endemic foci if high treatment coverage greater than 65% is maintained for a decade or more. Realistic estimates of the coverage levels needed to achieve elimination and eradication can inform donors' investment decisions. We estimated the number of treatments needed to go from the current control level to elimination and eradication of onchocerciasis as the geographic and therapeutic coverage is scaled up. Scenarios were developed assuming treatment continues until the population of female adult worms is reduced to a threshold where it is expected to irreversibly move to its demise. The number of treatments required, from 2012 to 2040, was predicted using historical data. The years of introduction and the coverage rates were collected from APOC treatment database, while for untreated areas they were predicted based on the expected launching year of APOC's budget plan, political and operational challenges, nodule prevalence, at-risk population, and average treatment coverage rates in the country. The treatment duration was predicted based on the existing results of a micro simulation model (ONCHOSIM). The estimated number of treatments needed in sub-Saharan countries is around 75 million in 2012. If the current strategy continues, the annual demand will increase to around 120 million by 2030. In the elimination scenario, the annual demand would decrease to around 30 million by 2030 because many endemic areas will not require treatment any longer. The treatment demand is expected to further decrease to less than 12 million by 2030 in the eradication scenario, whereby challenging areas with post-conflict situation or co-endemicity with loiasis will be treated with locally tailored approaches, so that treatment won't be required in the end. The results show that scaling up ivermectin coverage to achieve elimination and eradication would eventually lead to potential cost savings.

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WHAT HAVE WE LEARNED FROM GWAS STUDIES OF INSECTICIDE RESISTANCE IN *ANOPHELES GAMBIAE*?

David Weetman, Craig S. Wilding, Martin J. Donnelly

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Driven by advances in marker availability and screening technologies, the last six years has seen an explosion of genome-wide association studies (GWAS) in humans, most aimed at detecting genetic variants linked to common diseases. With a well-assembled genome, unquestioned medical

importance, and dwindling insecticide susceptibility a major threat to control, *Anopheles gambiae* insecticide resistance represented a natural target for the earliest GWAS in insects. Employing sequentially increasing numbers of markers, our studies have confirmed known causal variants and provided new field-applicable markers for pyrethroid resistance, but, in common with GWAS in humans, have yet to discover the novel variants of major effect that were initially anticipated. We highlight a number of design-related issues, recognition of which will aid future work. These include: (1) poor performance of designs inherited from human studies for mosquitoes and especially for insecticide resistance; (2) closer attention to phenotype definition, and (3) greater attention to environmental variation as a source of 'missing' trait heritability. With appropriate design alterations, GWAS can benefit considerably from imminent advances such as quality genome assemblies for multiple *Anopheles* disease vectors and a validated SNP-call database for *An. gambiae*.

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A SINGLE MUTATION IN THE GLUTATHIONE-S TRANSFERASE GENE (GSTE2) IS RESPONSIBLE FOR INSECTICIDE RESISTANCE IN THE MAJOR MALARIA *ANOPHELES FUNESTUS*

Jacob M. Riveron[1], Cristina Yunta[1], Sulaiman S. Ibrahim[1], Helen Irving[1], Rousseau Djouaka[2], Hanafy M. Ismail[1], **Charles Wondji**[1]

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

Metabolic resistance to insecticides is the biggest threat to the continued effectiveness of existing malaria vector control interventions. But its underlying molecular and genetic basis, crucial for successful resistance management, remains poorly characterised. In this study, using a genome-wide transcriptional analysis, we showed that the up-regulation of the glutathione-S transferase gene GSTe2 is strongly associated with DDT resistance. Using a GAL4/UAS transgenic expression of this gene in *Drosophila*, we demonstrated that over-transcription of this gene alone was necessary and sufficient to confer DDT resistance but more importantly also cross-resistance to pyrethroids. We showed that besides quantitative differences, qualitative changes in GSTe2 were also significantly contributing to the high DDT resistance as *In vitro* metabolic assays demonstrated that the resistant allele was more active in metabolizing DDT than the susceptible alleles. For the first time in mosquitoes, we identified an amino acid change (L119F) that strongly associates with DDT resistance and designed a molecular diagnostic assay that accurately detects the resistance in field populations. Structural analysis of the GSTe2 indicated that L119F located in the DDT-binding pocket confers the high DDT resistance by significantly increasing the size of the DDT binding cavity allowing more binding of the DDT molecule leading to its increased metabolism. The distribution of this L119F mutation across Africa shows a strong correlation with known patterns of DDT resistance. Furthermore, we showed that GSTe2 is under strong directional selection in resistant populations, and a restriction of gene flow is observed between African regions, enabling the prediction of the future spread of this resistance. This study represents a comprehensive and detailed dissection of the genetic, molecular and structural basis of metabolic resistance to insecticides and provides the first resistance marker for metabolic resistance in mosquitoes.

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INSECTICIDE RESISTANCE IN SYMPATRIC *ANOPHELES GAMBIAE* AND *AN. ARABIENSIS* FROM UGANDA: EVIDENCE FOR EVOLUTIONARY CONVERGENCE IN THE RESISTANCE ASSOCIATED GLUTATHIONE-S TRANSFERASE GSTE4

Craig S. Wilding[1], David Weetman[1], Henry D. Mawejje[2], Emily J. Rippon[1], Martin J. Donnelly[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]Infectious Diseases Research Collaboration, Kampala, Uganda

In Tororo, eastern Uganda, a high malaria transmission setting, and Jinja (approximately 110km from Tororo) where transmission intensity is lower, we have demonstrated extensive resistance to pyrethroid insecticides. This in the absence of universal distribution of ITNs or any IRS programme. In whole-genome microarray analysis of pyrethroid resistant *Anopheles gambiae* (Tororo) and *An. arabiensis* (Jinja) the glutathione-S transferase GSTe4 is significantly up-regulated in both species, suggestive of a role in the resistance phenotype. Where *An. gambiae* and *An. arabiensis* are sympatric we find a low level (0.22% *N*=7,202) of hybrid samples, and using a multiplex SNP array we demonstrate that in addition to F1s there are individuals which are the progeny of advanced backcrossing. This raised the possibility that introgression of selected genes, such as an insecticide resistance associated GSTe4 variant, may have occurred. Sequencing of GSTe4 haplotypes was not supportive of our hypothesis of introgression of GSTe4 variants but comparison of non-synonymous and synonymous changes were suggestive of marked functional constraints/ sequence convergence of GSTe4. Biochemical assays, showed that whilst GSTe4 does not actively metabolise pyrethroids, it is strongly inhibited by them, indicative that GSTe4 may play a role in sequestering insecticides in both species. This region of Uganda is experiencing marked flux in resistance status and species composition. We are using next generation whole genome sequencing of *An. gambiae* and *An. arabiensis* in order to better understand the consequences of hybridisation for the transfer of traits relevant to insecticide resistance between these important vectors.

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A UNIQUE MUTATION ON THE *ACE*-1 GENE OF THE MALARIA VECTOR *ANOPHELES ALBIMANUS* PROVIDES EVIDENCE FOR BALANCING SELECTION IN AN AREA OF HIGH INSECTICIDE RESISTANCE IN PERU

Kelly A. Liebman[1], Jesus A. Pinto[2], Jorge Valle[2], Lucrecia R. Vizcaino[1], William Brogdon[1], Audrey Lenhart[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Instituto Nacional de Salud, Lima, Peru

Acetylcholinesterase (AChE) insensitivity has previously been associated with resistance to organophosphate (OP) insecticides in arthropods. A single point mutation on the *ace-1* gene (G119S) has been identified in three anopheline species, including the New World malaria vector *Anopheles albimanus*. High levels of resistance to multiple classes of insecticides have recently been detected in the local *An. albimanus* vector population along the NW coast of Peru. To identify the mechanisms of resistance, the abdomens of 77 engorged females were excised and DNA was extracted, while the heads and thoraces of these individuals were used for biochemical analyses. Elevated levels of AChE insensitivity were detected in the biochemical assays, suggesting that this was a likely mechanism of resistance. A species-specific primer set was designed to amplify the region of the *ace-1* gene that includes the G119S mutation site. Sequencing the region showed that the individuals were highly polymorphic, with all individuals being heterozygous (G/T) at the first base. An additional, novel polymorphism was identified at the adjacent locus, where the individuals were all either heterozygous (G/C; n=63) or homozygous (C/C; n=14). The potential amino acids for individuals heterozygous at both *loci* are glycine (susceptible), serine (resistant), cysteine and alanine. For homozygous individuals at the second base, the only potential amino acids are serine and alanine, suggesting this

novel substitution may be associated with greater AChE insensitivity. This hypothesis is supported by analysis of biochemical and genetic data from the same individuals, which indeed suggests that individuals homozygous at base two presented higher levels of AChE insensitivity than heterozygotes. The G119S mutation appears to have arisen independently in this population, as the polymorphisms that result in serine are unique to what has been previously described. The occurrence of heterozygotes at 2 *loci* suggests that balancing selection could be the driving force behind the maintenance of OP resistance in this population.

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INSECTICIDE RESISTANCE SELECTION DRIVES GENETIC DIFFERENTIATION AMONG *AEDES AEGYPTI* FROM YUCATAN

Karla L. Saavedra-Rodriguez[1], Meaghan Beaty[1], Steven Denham[1], Saul Lozano-Fuentes[1], Julian Garcia-Rejon[2], Guadalupe Reyes-Solis[2], Carlos Machain-Williams[2], Maria A. Lorono-Pino[2], Barry Beaty[1], Lars Eisen[1], William C. Black, IV[1]

[1]Colorado State University, Fort Collins, CO, United States, [2]Universidad Autonoma de Yucatan, Merida, Mexico

The mosquito *Aedes aegypti* is the main vector of dengue viruses. Population reduction involves removal of larval breeding sites and uses insecticides for larval and adult control. In Mexico, permethrin has been used for mosquito control over the last 12 years and widespread resistance has been reported. Knockdown and mortality rates obtained by cone assay were highly variable among Yucatan collections and are highly correlated with point mutations in the voltage gated sodium channel gene (VGSC). We sought to determine if permethrin pressure reduces gene flow by comparing SNP variation at neutral gene markers and with variation at markers putatively associated with insecticide resistance. We tested for patterns of gene flow among 27 collections from Yucatan made in 2011. Three groups of nested collections were made around Merida and five collections were in towns outside Merida. A total of 1,301 mosquitoes were genotyped using 13 single nucleotide polymorphism markers (SNPs). Eight SNP's were in putatively insecticide neutral genes: amylase, apyrase, gluco-phosphate isomerase, early trypsin, vitellogenic carboxypeptidase precursor, chymotrypsin and maltase. Two SNPs were in the VGSC gene (C1534 and I1016), two were in cytochrome P_{450} genes (CYP9J32 and CYP9J29) and one was in a carboxyl/choline esterase gene (CCEae1C). F_{ST} for neutral SNP's was low (0.012 - 0.063) and F_{ST} for potential insecticide metabolic genes were similar (0.031- 0.049). However, F_{ST} for SNP's at the VGSC were much higher (0.222 and 0.135 for C1534 and I1016, respectively). AMOVA among all *loci* indicated little variation (3%) among collections from different cities. However, locus by locus analysis showed that C1534 and I1016 cause 22 and 13% of the variation among cities, respectively. In the face of high effective migration rates, local insecticide selection pressure created large variation in VGSC mutations.

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THE RAPID SELECTION OF PYRETHROID RESISTANCE IN *ANOPHELES GAMBIAE* IN A SINGLE YEAR: AN INVESTIGATION INTO THE UNDERLYING CAUSES AND POTENTIAL IMPACT

Hyacinthe K. Toe[1], Christopher M. Jones[2], N'Fale Sagnon[1], Moussa W. Guelbeogo[1], Moussa W. Guelbeogo[1], Antoine Sanou[1], Roch K. Dabire[3], Hilary Ranson[2]

[1]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso, [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [3]IRSS/Centre MURAZ, Bobo-Dioulasso, Burkina Faso

Resistance to pyrethroids was first reported in *Anopheles gambiae* from the rice growing region of Vallee de Kou in Burkina Faso over 14 years ago. The proportion of resistant mosquitoes has steadily increased since then and it is now rare to find any mosquitoes from this region surviving standard WHO susceptibility diagnostic dose assays. However, without data on the magnitude of resistance it is difficult to predict the impact that this may have on malaria vector control. To address this, we determined the LT50 of the predominant vector from Vallee de Kou, An *gambiae* M form, in 2011 and again in 2012. Remarkably, in just one year, the LT50 had increased > 10 fold. This dramatic increase in the strength of resistance was not accompanied by an increase in kdr frequency, as the frequency of the 1014F allele was >0.8 in both years and the 1575Y allele decreased slightly between the years to 0.27 in 2012. However, using a stringent microarray experiment, with comparisons with multiple susceptible strains, we identified a number of detoxification genes strongly correlated with resistance to deltamethrin. Expression of a subset of these genes, including cytochrome P450 and cuticular genes increased significantly between 2011 and 2012 and may explain the dramatic increase in resistance observed recently. Data on the impact of this very strong resistance phenotype on the efficacy of long lasting insecticide treated bed nets in use in the region, obtained using both cone bioassays and experimental huts will also be presented.

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DISSEMINATION OF A POTENT PUPACIDE BY ADULT *AEDES AEGYPTI* UNDER FIELD CONDITIONS: MECHANISMS OF A POTENTIAL CONTROL TOOL

Gregor John Devine[1], Elvira Zamora Perea[2], Nicole Achee[3]

[1]Tropical Public Health Services, Cairns, Australia, [2]Instituto Leonidas e Maria Deane, Manaus, Brazil, [3]Uniformed Services University of the Health Sciences, Bethesda, MD, United States

Recent studies show that the behaviour of adult mosquitoes can be exploited for the dissemination of insecticides to aquatic habitats. This requires further characterization and optimization if it is to be widely adopted. A "lure and disseminate" device was designed that 1) contaminated wild mosquito populations with a potent pupacide and 2) released those mosquitoes to auto-disseminate pupacide to larval habitats. These dissemination tools were deployed in the field in Iquitos, Peru. Using coloured markers, the patterns and mechanics of dissemination between lures and sentinel oviposition habitats were examined. The potential contamination of the adult population was high and mark-recapture data revealed an even distribution of contaminated mosquitoes among sentinel aquatic habitats and a high frequency of contamination events at those habitats (ca 1 event every 4 days). Male mosquitoes, and other mosquito genera (particularly *Culex* spp) contributed to the dissemination process. The coloured markers were replaced with finely-milled pyriproxyfen (PPF) granules, and the impact of its subsequent dissemination by dispersing mosquitoes was assessed. Variation in juvenile mortality between aquatic habitats and trials was large (0-100%) but when all trials were averaged, 85% of sentinel larvae failed to develop. More than 75% of deaths occurred at the pupal stage. Affected aquatic habitats retained their pupacidal impact for 4 days after the contaminating devices had been removed from the trial site. The exposure of adult females to PPF using these dissemination tools also affected their reproductive potential. Only 46% of eggs laid at sentinel oviposition sites hatched. In contrast, 97% of eggs laid during control periods eclosed. These field results provide an essential understanding of the factors driving the remarkable efficacy of the auto-dissemination technique. These include 1) potency of the pupacide, 2) precise targeting of the insecticide by the mosquito, 3) amplification in coverage between the contaminating tools and the aquatic habitat, 4) persistence of the pupacide and 5) pupacides are unaffected by density dependent processes in the aquatic environment. The auto-dissemination technique, demonstrated here using a standardized contamination tool, a WHO-recommended pupacide, and a naturally-occurring mosquito population has enormous potential.

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CHARACTERIZATION OF STRAIN-SPECIFIC EFFECTS ON TRANSMISSION AND MAINTENANCE OF WEST NILE VIRUS

Alexander T. Ciota, Greta A. Van Slyke, Dylan J. Ehrbar, Kiet A. Ngo, Laura D. Kramer

Wadsworth Center, New York State Department of Health, Slingerlands, NY, United States

West Nile virus (WNV; *Flaviviridae, Flavivirus*) cases in New York State (NYS), as well as nationwide, were historically high in 2012. In addition, maximum likelihood estimates based on testing of NYS mosquito pools demonstrated the highest prevalence in *Culex* mosquitoes since the introduction of WNV to NYS, with approximately 7.5 WNV-positive *Culex* per 1000 tested. Although environmental factors are likely important in driving epidemiological shifts, the role of both WNV consensus and intrahost genetic variation in governing temporal shifts in vectorial capacity has not been adequately assessed. In addition, variation in the capacity for vertical transmission and, therefore, overwintering success, has not been evaluated. Previous studies demonstrate that WNV effects on *Culex* life-history traits are strain-specific, establishing the need to evaluate factors beyond vector competence, including strain virulence in mosquitoes and alteration to both bloodfeeding and reproductive patterns, in order to accurately measure strain-specific differences in transmissibility and maintenance. To begin to evaluate these relationships, we used deep-sequencing to genetically characterize WNV strains isolated from *Culex* pools in Suffolk County, NY in both 2005 and 2012, representing low and high activity years, respectively, and performed subsequent phenotypic analyses including quantifying vector competence, life-history traits following exposure, and vertical transmission. Genetic analyses suggest consensus substitution rates of approximately 6 X 10^{-4}/base/year, which is comparable to what has been measured in previous studies, yet identification of single nucleotide polymorphisms (SNPs) demonstrate substantial differences in mutant swarm breadth between isolates, with 19 minority SNPs identified in a 3kb region of the WNV 2005 genome, relative to 9 for the WNV 2012 isolate. Preliminary studies also demonstrate differences in infectivity in *Cx. pipiens*; and initial assessment of life-history traits following exposure suggests potentially important strain-specific effects. Taken together, these data begin to inform our understanding of the relationship between WNV genetic variation and temporal fluctuation in WNV activity.

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NS3-249 AMINO ACID SUBSTITUTIONS ALTER AVIAN PATHOGENESIS OF BOTH LINEAGE 1 AND 2 WEST NILE VIRUSES

Aaron C. Brault[1], Angela Bosco-Lauth[1], Michael Anishchenko[1], Stanley A. Langevin[2], Christy C. Andrade[2], Joanie L. Kenney[1], Hannah Romo[3], Anna Papa[4], Richard A. Bowen[3]

[1]Centers for Disease Control and Prevention, Fort Collins, CO, United States, [2]University of California, Davis, CA, United States, [3]Colorado State University, Fort Collins, CO, United States, [4]Aristotle University of Thessaloniki, Thessaloniki, Greece

Previous studies using selection modeling and experimental avian inoculations identified a single West Nile viral genetic *loci* (NS3-249) of lineage 1 WNVs to be under the effect of positive selection and to be a virulence determinant (NS3-249P) associated with increased replicative capacity and virulence in American crows (AMCRs), a sentinel species utilized in North America to track the spread of the virus. Although a lineage 2 virus was isolated from a moribund goshawk in Hungary in 2004, viruses from this lineage have not been associated with significant avian mortality. All genetically characterized lineage 2 viruses have been identified to have a His at the AMCR virulence locus, NS3-249; however, WNV isolates made from a large lineage 2 WNV outbreak in Greece in 2010 were identified to have a Pro at this site. A WNV infectious cDNA of a South African strain isolated in 1989 was subsequently generated and an NS3-H249P mutation incorporated to assess the potential modulatory effect of this locus in an alternative WNV lineage. Inoculation of AMCRs with the parental South African or cDNA clone-derived virus demonstrated mean peak viremias of 7-7.5 $\log_{10}$ PFU/mL sera and exhibited approximately 30% mortality. In contrast, the NS3-H249P lineage 2 mutant virus demonstrated 100% mortality in AMCRs with an approximate 100-fold higher mean peak viremia (9.6 $\log_{10}$ PFU/mL sera), indicating the potential importance of this specific genetic alteration within the lineage 2 genome for eliciting high replicative capacity in avian hosts. These results confirm the vital role of this locus for avian virulence potential and indicate the selective advantage of different NS3-249 residues for increased avian replication within both lineage 1 and lineage 2 WNV genetic backbones.

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SEQUENCE AND PHENOTYPIC ANALYSES OF 2012 WEST NILE VIRUS ISOLATES FROM TEXAS FAIL TO ASSOCIATE VIRAL GENETIC FACTORS WITH OUTBREAK MAGNITUDE

Nisha Duggal, Roger Nasci, Aaron C. Brault

Centers for Disease Control and Prevention, Fort Collins, CO, United States

In 2012, the U.S. experienced the largest outbreak of WNV human encephalitis since 2003. In order to determine whether the increase in WNV transmission in 2012 could have been due to recent sequence changes in the WNV genome, we sequenced 17 full-length isolates made from mosquito pools in Texas in 2012 and compared them to isolates from previous years. We found a similar amount of divergence in the 2012 Texas isolates compared to isolates from previous years, with most of the genome evolving under purifying selection and genetic drift. Further, we compared isolates from Dallas County, that exhibited a 2012 incidence rate of 16 WNV cases per 100,000 population, to isolates from Montgomery County, with a 2012 incidence of 3 WNV cases per 100,000 population. While genetic differences did exist between Dallas and Montgomery County viral populations, weak evidence supports genetic population subdivision or adaptive changes in the Texas isolates. Finally, *in vitro* growth rates of Dallas and Montgomery County WNV isolates with the aforementioned genetic differences were assessed in mammalian and mosquito cells. Results demonstrated that isolates with variable amino acids exhibited indistinguishable replication profiles compared to one another or to the NY99 strain, indicating that these 2012 WNV genetic differences did not afford an *in vitro* replication advantage. Together, these data do not support genetic viral adaptation as an explanation for increased WNV incidence in 2012.

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SMALL RNA RESPONSE OF *CULEX QUINQUEFASCIATUS* TO WEST NILE VIRUS INFECTION: RELATIONSHIP TO VECTOR COMPETENCE

Abhishek N. Prasad[1], Darci R. Smith[1], Doug E. Brackney[1], Benjamin Dodd[1], Thomas D. Harrison[1], Corey L. Campbell[1], Jennifer E. Beane[2], Gregory D. Ebel[1]

[1]Colorado State University, Fort Collins, CO, United States, [2]Boston University, Boston, MA, United States

Culex mosquitoes are among the most important vectors of animal viruses worldwide. These include West Nile virus, Japanese Encephalitis virus, Rift valley fever virus and others. However, our understanding of the molecular events that influence their ability to transmit pathogens (vector competence) is incomplete. Variation in vector competence occurs between individual mosquitoes, between populations of the same species, and between taxonomically distinct species. When exposed to the same virus-containing bloodmeal, some mosquitoes fail to become infected, others become infected but limit virus replication and dissemination, while others develop disseminated infection and ultimately transmit virus. Vector competence has been shown through several studies to be a quantitative trait under the control of several genes and other factors. RNAi is widely

regarded as the most important antiviral pathway in mosquitoes, but its role in shaping mosquito vector competence is poorly understood. Moreover it is not clear how RNAi influences vector competence in non-transgenic "wild-type" vector mosquitoes. Therefore, we sought to characterize the mosquito RNAi response to WNV infection and determine its influence on vector competence using colonized *Cx. quinquefasciatus* mosquitoes. Mosquitoes were exposed to WNV in an artificial bloodmeal and held for various extrinsic incubation periods. Small RNA (sRNA) profiles were obtained using next-generation sequencing. To characterize the early sRNA responses to WNV, midguts were removed from mosquitoes 12 and 24 hours after feeding and sRNAs mapped to the WNV genome. To assess the relationship between sRNA responses and virus dissemination from the midgut (a prerequisite for virus transmission), midguts and legs were removed from mosquitoes at 7 and 14 days post feeding. sRNA responses from mosquitoes that permitted WNV dissemination from the midgut into peripheral tissues were compared to those with WNV limited to the midgut. Overall, these studies will characterize the sRNA responses of mosquitoes to WNV infection and determine the extent to which RNAi influences vector competence in this system.

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EVALUATION OF CHIMERIC JAPANESE ENCEPHALITIS VIRUS/ DENGUE VIRUS TYPE 4 VACCINE CANDIDATES IN MICE

Gregory D. Gromowski, Cai-Yen Firestone, Christopher T. Hanson, Stephen S. Whitehead

National Institutes of Health, Bethesda, MD, United States

Japanese encephalitis virus (JEV) is a leading cause of viral encephalitis worldwide and vaccination is one of the most effective ways to prevent disease. A suitable live attenuated JEV vaccine could be formulated with a live attenuated tetravalent dengue vaccine for the control of these viruses in endemic areas. Toward this goal, we previously generated chimeric vaccine candidates by replacing the precursor membrane (prM) and envelope (E) structural genes of dengue virus type 4 (DEN4) or attenuated DEN4Δ30 with those of JEV India/78. These first generation JEV/DEN4 chimeric viruses were attenuated for neurovirulence and neuroinvasiveness in weanling mice compared to the wild-type JEV parent, which warranted their further development as vaccine candidates. Adventitious mutations in E, NS3 and NS4B proteins that arose during adaptation of first generation chimeric viruses for replication in Vero cells were engineered into a second generation of JEV/DEN4 chimeric viruses. Novel 3'UTR deletions, similar to those found in DEN4Δ30, were also introduced. Sequencing revealed that chimeric viruses lacking engineered Vero cell adaptive E protein mutations acquired adventitious mutations. This suggests that at least one adaptive E protein mutation is required for genetic stability of JEV/DEN4 chimeric viruses propagated in Vero cells. The second-generation chimeric viruses were attenuated for neurovirulence and neuroinvasiveness in weanling mice. They were also significantly more attenuated for neurovirulence in suckling mice than the wild-type JEV parent and the JEV SA14-14-2 live-attenuated vaccine strain, based on LD50 values and survival times. Deletions in the 3'UTR also increased attenuation for suckling mice. By contrast, a single E protein mutation that is shared by JEV SA14-14-2 significantly increased neurovirulence in suckling mice and replication in Vero cells for one chimeric virus. We are currently evaluating these chimeric vaccine candidates in mice for immunogenicity and protection from challenge with wild-type JEV.

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ESTIMATING THE BURDEN OF YELLOW FEVER IN AFRICA

Tini Garske[1], Maria D. Van Kerkhove[1], Sergio D. Yactayo[2], Olivier Ronveaux[3], Rosamund F. Lewis[4], William Perea[2], **Neil M. Ferguson**[1], na Yellow Fever Expert Committee[2]

[1]Imperial College, London, United Kingdom, [2]World Health Organization, Geneva, Switzerland, [3]World Health Organization, Ouagadougou, Burkina Faso, [4]Ottawa Public Health, Ottawa, ON, Canada

Yellow fever is a vector-borne disease affecting humans and non-human primates in tropical areas of Africa and South America. While eradication is not possible due to the wildlife reservoir, large scale vaccination activities in Africa in the 1940s to 1960s reduced yellow fever incidence for several decades. However, after a period of low vaccination coverage, yellow fever has resurged in the continent. Since 2006 there has been substantial funding for preventive mass vaccination campaigns for yellow fever in the most affected countries in Africa to curb the rising burden and control future outbreaks. Generalised linear regression models were fitted to a dataset of the locations of yellow fever outbreaks in the last 25 years to estimate the probability of outbreak reports across the endemic zone. Environmental variables and indicators of surveillance quality in the affected countries were used as covariates. By comparing probabilities of outbreak reports estimated in the regression with the force of infection estimated for a limited set of locations for which serological surveys were available, the detection probability per case and the force of infection were estimated across the endemic zone. The yellow fever burden in Africa was estimated for the year 2013 as 130,000 (95% CI 84,000 - 170,000) severe cases including 44,000 (95% CI 29,000 - 60,000) deaths, taking into account the current level of vaccination coverage. The recent mass vaccination campaigns are estimated to have reduced this burden by 27% (95% CI 23 - 30%) across the region, achieving up to 82% reduction in countries targeted by these campaigns. With the estimation method presented here, spatial estimates of transmission intensity can be combined with vaccination coverage levels to evaluate the impact of past or proposed vaccination campaigns, thereby helping to allocate resources efficiently for yellow fever control. *Expert Committee: Donald Burke, Fernando De La Hoz, Bryan Grenfell, Peter Hansen, Raymond Hutubessy, Rosamund Lewis, William Perea, Olivier Ronveaux, Erin Staples, Sergio Yactayo.

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ISOLATION AND CHARACTERIZATION OF PARAISO ESCONDIDO VIRUS: A NEW FLAVIVIRUS *IN LUTZOMYIA (PSATHYROMYIA) ABONNENCI* SANDFLIES FROM ECUADOR

Cigdem Alkan[1], Sonia Zapata[2], Laurence Bichaud[1], Gregory Moureau[1], Gregory Moureau[1], Xavier de Lamballerie[1], Jerome Depaquit[2], **Remi N. Charrel**[1]

[1]Aix Marseille University - IRD French Institute of Research for Development - EHESP French School of Public Health, Marseille, France, [2]Université de Reims Champagne-Ardenne - ANSES, Reims, France

Flaviviruses consist of mosquito-borne, tick-borne, insect-only and non-vectored viruses. Sandflies are not recognized as principal vectors of flaviviruses. We report here the discovery (detection, isolation and full-length sequence) of a novel flavivirus in *Lutzomyia (Psathyromyia) abonnenci* that was provisionally named Paraiso Escondido virus. Twenty six pools of *Lutzomyia* flies were screened for the presence of flaviviruses. One pool of female (neither gravid nor engorged*) Lutzomyia (Psathyromyia) abonnenci* was found to contain flavivirus RNA through real time RT-PCR assay targeting all flaviviruses, as previously reported. Assuming that one sandfly only was infected in the pool, quantitative real-time PCR estimated that > 1012 genome copies were in the infected insect individual. Virus isolation was obtained in C6/36 cells. The complete genome was sequenced using next generation sequencing technology based on Ion-torrent PGM. The genome consisted of 10,760 nucleotides encoding 3441 AA with 5'- and 3'-UTR of 119 and 316 nts,

respectively. A series of cysteine residues and potential glycosylation sites were identified. The enzymatic domains (serine-protease, helicase/NTPase, methyltransferase and RNA-dependent RNA polymerase) of Paraiso Escondido virus were found to be highly conserved in comparison with other flaviviruses. The putative cleavage sites of the polyprotein were identified and found substantially different from those of other flaviviruses. The AA distances observed ranged 53-85%, 40-72%, 35-56% with envelope, NS3 and NS5 proteins. Phylogenetic analyses based on amino acid alignments showed that Paraiso Escondido virus clusterized together with *Aedes*-borne flaviviruses although it is clearly distinct from other known flaviviruses. In the New world, *Lutzomyia* sandflies are the vectors of viruses (vesicular stomatitis virus, Orbivirus, Punta Toro virus), parasites (leishmaniasis) and bacteria (bartonellosis). Therefore they should be considered as possible vectors of viruses of potential medical and veterinary importance. Further investigations are on-going to determine whether Paraiso Escondiso virus is capable to infect vertebrates and humans.

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INTEGRATED, COMMUNITY-BASED SURVEYS OF INTESTINAL PARASITIC INFECTIONS WITH TRACHOMA IMPACT ASSESSMENTS IN AMHARA NATIONAL REGIONAL STATE, ETHIOPIA

Tekola Endeshaw[1], Woyneshet Gelaye[2], Elisabeth Escher[3], Aisha P. Stewart[4], Genetu Alemtaye[2], Sileabatt Melaku[2], Zerihun Tadesse[1], Peter Odermatt[3], Jürg Utzinger[3], Paul M. Emerson[4], **Jonathan D. King**[4]

[1]The Carter Center, Addis Ababa, Ethiopia, [2]Amhara Regional Research Laboratory, Bahir Dar, Ethiopia, [3]Swiss Tropical and Public Health Institute, Basel, Switzerland, [4]The Carter Center, Atlanta, GA, United States

In the Amhara National Regional state of Ethiopia we integrated assessment of intestinal parasitic infections into large-scale trachoma impact surveys to establish baseline prevalence upon which to monitor the impact of integrated control measures of improved hygiene, water, sanitation, and preventive chemotherapy. Both trachoma and intestinal parasites (*Schistosoma mansoni*, soil-transmitted helminths, and intestinal protozoa) were assessed in systematically selected clusters from a geographic listing of communities by district. One child aged 6-15 years per household in selected clusters was randomly selected to provide a stool sample of which about 1 g was preserved in sodium acetate-acetic acid-formalin, processed using formol-ether concentration and examined under a microscope by experienced laboratory technicians. A total of 6,732 stool specimens were collected from 368 communities. The prevalence of *S. mansoni* was 6.6% (range by district 0-40.9%), but prevalence in 55 communities was ≥10%. The overall prevalence of any soil-transmitted helminth infection was 22.5% (range by district 3.0-77.7%). Approximately 3 in 4 children were infected with at least one intestinal protozoa. The prevalence of Giardia intestinalis was 18.9% (range by district 5.4-41.0%) and Entamoeba histolytica/E. dispar was 12.5% (range by district 2.9-22.5%). Associations between soil-transmitted helminth infections and community-level indicators of hygiene, water, and sanitation were explored. According to World Health Organization guidelines, preventive chemotherapy targeted to school-aged children is warranted for the control of schistosomiasis in 10 and for the control of soil-transmitted helminths in 39 out of 59 districts. Integration of deworming with mass distribution of antibiotics for trachoma might further expand health benefits to co-endemic communities. Integrating assessment of intestinal parasitic infections with community-based trachoma prevalence surveys may be a feasible method for evaluating impact of neglected tropical disease control programs.

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INTEGRATED SCHOOL-BASED SURVEILLANCE FOR SOIL-TRANSMITTED HELMINTH INFECTIONS AND FOR LYMPHATIC FILARIASIS IN GAMPAHA DISTRICT, SRI LANKA

Nipul K. Gunawardena[1], Sharmini Gunawardena[2], Ganga Kahathuduwa[3], Nadira D. Karunaweera[2], Nilanthi de Silva[1], Udaya S. Ranasinghe[3], **Ramakrishna U. Rao**[4], Maria Rebollo[5], Gary J. Weil[4]

[1]University of Kelaniya, Ragama, Sri Lanka, [2]University of Colombo, Colombo, Sri Lanka, [3]Antifilariasis Campaign, Ministry of Health and Nutrition, Colombo, Sri Lanka, [4]Washington University School of Medicine, St. Louis, MO, United States, [5]Centre for Neglected Tropical Diseases, The Liverpool School of Tropical Medicine, Liverpool, United Kingdom

The Sri Lankan Anti-Filariasis Campaign (AFC) conducted 5 rounds of annual mass drug administration (MDA) with albendazole and DEC in 2002-2006 in 8 districts that were endemic for lymphatic filariasis (LF) (target population approximately 10 million). AFC conducted transmission assessment surveys (TAS) in 2012, about 6 years after the last round of MDA. This study explored the practicality of integrating surveillance for soil transmitted helminth (STH) infections with TAS for LF in Gampaha district (population 2.3 million). The district was divided into two Evaluation Units (EUs), coastal and inland. Each TAS tested 1st and 2nd grade school children drawn from 30 randomly selected schools (N=1,462 inland, 1,642 coastal). Tests included the ICT card test for filarial antigenemia (performed by AFC personnel) and the Kato-Katz test for detection of STH ova (performed by university personnel). ICT rates were 0% and 0.1% (0.01-0.3% CI) in the inland and coastal EUs, respectively. These results suggest that LF transmission rates are very low in Gampaha District. The STH survey was conducted at the same time as the TAS in the inland EU (955 stools from 1,211 children) and several weeks after the TAS in the coastal EU (927 stools from 1,586 children). STH infection rates and stool sample participation rates were 0.8% and 79% in the inland EU and 2.8% and 58% in the coastal EU. Most of the STH infections detected were low-intensity*Trichuris* (present in 73% of positive stools). The low STH rates are probably due to the country's national school deworming program (mebendazole in grades 1, 4, and 7) and relatively good sanitation in Gampaha district. The cost for STH testing was approximately $5,000 per EU. These results suggest that it is feasible for national NTD programs to integrate school based surveillance for STH and LF. Further work is needed to streamline procedures and to determine optimal sampling strategies for STH surveys, because these may not require as many samples or sampling sites as TAS.

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QUANTIFYING THE QUALITY OF SURVEY DATA FOR THEIR USE IN THE DESIGN OF SOIL TRANSMITTED HELMINTH AND SCHISTOSOMIASIS CONTROL PROGRAMS

Birgit Nikolay[1], Charles S. Mwandawiro[2], Sammy M. Njenga[2], Rachel L. Pullan[1], Simon J. Brooker[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Kenya Medical Research Institute, Nairobi, Kenya

Generous medication donations and increasing political commitment has led to the launch of numerous national neglected tropical diseases (NTD) control programmes. The first step in developing these programmes is often country-wide mapping of disease, which might be unnecessary if data from previously conducted studies exist. Thus, large scale surveys would waste resources and cause unnecessary delays for medication distribution. As the quality of previously collected data can vary in terms of study design and data collection, we developed guidelines for the use of available soil transmitted helminth (STH) and schistosomiasis survey results to support programme implementers during the process of programme design. These guidelines allow identifying areas where sufficient information already exists and others that should be prioritised for surveys. The approach is based on three steps: i) the identification of ecological

zones within the country, ii) the identification and classification of available surveys and iii) the classification of each ecological zone based on characteristics of individual surveys. The country is divided into ecological zones based on environmental data and a systematic literature research is performed to capture all relevant studies. The quality of the identified surveys is assessed based on the following characteristics: We considered the time since surveys, as well as expected substantial changes in transmission. Additionally, the representativeness of the study population is taken into account, as well as the sampling design and sample size. The accuracy of diagnostic methods is graded based on published comparative studies of diagnostic tools. Furthermore, the level of available information is assessed in terms of geographic information (precise locations of surveys vs. district or province summary estimates) and infection information (species specific prevalence vs. STH summary estimates). According to the coverage and quality of identified surveys, each ecological zone is classified independently into five groups i) mapped as recommended, ii) mapped, iii) mapped with low quality data, iv) mapped with questionable data, and v) not mapped. Based on the grading of ecological zones, we further provide recommendations for the design of national surveys. Finally, we demonstrate the application of the proposed guidelines on the example of Kenya and discuss their potential constraints.

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TREATMENT COVERAGE OF INTEGRATED MASS DRUG ADMINISTRATION FOR NEGLECTED TROPICAL DISEASES IN TOGO

Monique A. Dorkenoo[1], **Rachel N. Bronzan**[2], Kwame C. Amlaga[3], Michel G. Datagni[3], Kangni Adadé[4], Touka M. Djato[4], Boakye A. Boatin[5], Koffi S. Sognikin[6], Kodzo A. Anthony[3]

[1]Lymphatic Filariasis Programme, Ministry of Health, Lomé, Togo, [2]Health and Development, International, Rockville, MD, United States, [3]Health and Development, International, Lomé, Togo, [4]Onchocerciasis Control Programme, Ministry of Health, Lomé, Togo, [5]Lymphatic Filariasis Support Centre, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana, [6]Neglected Tropical Diseases Program, Lomé, Togo

Since 2009, the country of Togo has implemented a program for the integrated control of neglected tropical diseases. Under this program, onchocerciasis, schistosomiasis, and soil transmitted helminths are targeted using community-based distribution of ivermectin (IVM), praziquantel (PZQ), and albendazole (ALB). Drugs are given to selected populations based on local prevalence of each disease. A nationwide integrated mass drug administration (MDA) was conducted in July 2012; while reported treatment coverage was high, integrated MDA campaigns are logistically complex and reported coverage may not reflect the actual coverage achieved. In November 2012, Togo conducted a survey to validate coverage for all three diseases. Four cluster surveys were conducted, one in each of three geographically disparate districts, and a fourth in areas with high prevalence of onchocerciasis. In each district 30 to 45 villages were selected with probability proportional to size. Ten houses were selected in each village and all household members were asked about receipt of drugs in July 2012. Each household head answered questions about knowledge of the diseases. In the four clusters a total of 9511 persons in 1187 households were interviewed. Coverage varied by district: 74-84% of the population received IVM, 80-94% of school-age children (SAC) received PZQ, and 84-94% of SAC received ALB. Measured coverage for PZQ and ALB exceeded WHO targets; coverage for IVM was below WHO target in two districts. In one district measured coverage was lower than reported for all drugs ($P<0.05$), and in two others measured coverage was lower than reported for ALB. Coverage validation surveys are an important part of program evaluation. The sampling for this survey was challenging and novel since the targeted diseases and populations vary by village and no clear guidelines exist for sampling in such a complex distribution scheme. Results from this study will be used to refine MDA training and supervision, and will also contribute to decisions regarding control and/or elimination strategies for these diseases.

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"COORDINATED" MAPPING FOR NEGLECTED TROPICAL DISEASES IN COTE D'IVOIRE

Anna E. Phillips

Imperial College, London, United Kingdom

Where geographic overlap among NTD distribution exists, coordinated mapping could result in significant resource savings. There are few data on the geographic distribution of NTDs in post-conflict Cote D'Ivoire. To guide intervention, by the recently established national Lymphatic Filariasis, Schistosomiasis and Soil Transmitted Helminth Control Programme, a coordinated prevalence survey for schistosomiasis, soil-transmitted helminth (STH) infection and lymphatic filariasis (LF) was conducted. The aim was to design a resource efficient protocol to establish which communities required mass drug administration (MDA), according to World Health Organization thresholds. The sampling frame was the health district with a total of eight health districts sampled. Within each health district 20 communities were surveyed for schistosomiasis and STH, sampling 50 school-age children per village. Among the 20 communities selected, two were also surveyed for LF with 100 adults sampled in each. In total 8,000 school-aged children were tested for both urinary and intestinal schistosomiasis and STH. A further 1600 adults were tested for circulating *Wuchereria bancrofti* antigen using immunochromatographic card tests (ICT). Preliminary analysis has shown prevalence of *Schistosoma haematobium* and *S. mansoni* ranged from 2-69% and from 0-76%, respectively. The main STH species was hookworm, ranging from 2-41% by village. LF and cost-analysis results are under-going preliminary analysis. This was the first attempt at using a coordinated survey design for this group of infections in Cote D'Ivoire. The approach proved practical and the results show that only a few areas need to be targeted with MDA, thus confirming the importance of detailed mapping for cost-effective control.

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BARRIERS TO COMPLIANCE WITH MASS DRUG ADMINISTRATION FOR NTD CONTROL PROGRAMS

Achille Kabore, Philip Downs

RTI International, Washington, DC, United States

Compliance with mass drug distribution is a determinant of success for national neglected tropical diseases (NTD) control programs. Persistent non-compliance can maintain diseases transmission as a reservoir for potential re-infection. Out-of school children, women of reproductive age and hard-to-reach remote populations might repeatedly miss opportunities for mass drug distribution (MDA); creating systematic non-compliant groups for preventive chemotherapy. An analysis of semi-annual reports and post MDA coverage surveys from the USAID funded NTD control programs and other NTD projects was conducted to identify barriers to adhering to MDA treatment schedules and keys factors amenable to corrective measures. The review identified several groups of individuals including institutionalized people, non-enrolled school age children, and people from high socio-economic status as persistently non-compliant during MDAs. Also identified were 47% of women excluded from MDA due to pregnancy or nursing of babies under 1 month of age, and who later qualify for treatment but did not attend consecutive rounds of annual MDAs. Large sections of urban populations in endemic settings remain consistently untreated because they present specific challenges in terms of acceptability of drugs distributed by non health professionals. Other reasons for recurrent non participation in mass campaigns include: 1) fear of adverse event, 2) non perceived benefit, and 3) lack of disease awareness. The authors also explore campaign fatigue resulting from the long standing drug distribution programs. Strategies to overcome barriers and to increase compliance involve refinement of pre-MDA plans, comprehensive registration of all eligible populations, intensive IEC campaigns, implementation of flexible distribution mechanisms, and routine facility-based post campaign drug administration. The authors

recommend adapting MDA to the local environment and making use of platforms and local opportunities to increase NTD programs visibility and drug coverage.

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MONITORING AND EVALUATING INTEGRATED NTD CONTROL: FIVE-YEAR IMPACT OF TREATMENT ON INFECTION IN NIGER AND BURKINA FASO

Anna E. Phillips

Schistosomiasis Control Initiative, Imperial College, London, United Kingdom

Monitoring and evaluation (M&E) is an essential element of national NTD control that guides a programme on how to strengthen its approach. Standard epidemiological monitoring methods, used to measure the impact of treatment through annual parasitological examinations at school sentinel sites, are being employed across all SCI countries. The findings from two countries are presented here. A longitudinal cohort of 718 and 2405 children aged 7-12 years in Niger and Burkina Faso, respectively, were recruited at baseline (2004) and parasitological examinations carried out at yearly intervals before and after large-scale treatment for schistosomiasis and STH. Preventative chemotherapy (PCT) was integrated against five NTDs (lymphatic filariasis, schistosomiasis, STH, onchocerciasis and trachoma) in 2007. In order to monitor the impact of combined mass drug administration, integrated (schistosomiasis, STH, and trachoma) sentinel schools were added in 2008. Data from the longitudinal cohort demonstrated that a significant decrease in the odds of detectable trachoma, as well as *Schistosoma haematobium* infection, was found at follow-up two years post-baseline. Children who benefited most from anthelmintic treatment, in terms of increased haemoglobin concentrations, were those who had presence of anaemia and highly positive microhaematuria scores at baseline. This study demonstrates that chemotherapy can have a substantial impact on both S.*haematobium* and trachoma infection, and its associated morbidity in children, even after integrating PCT for several NTDs. These are the first known integrated sentinel sites to examine all three NTDs, the results of which will demonstrate whether the presence of co-infection affects the impact of treatment as well as the cost-efficiency of combining M&E for multiple infections.

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THE UTILITY OF DIAGNOSTIC TESTS FOR TYPHOID FEVER AT CHITTAGONG MEDICAL COLLEGE HOSPITAL (CMCH), CHITTAGONG, BANGLADESH

Rapeephan R. Maude[1], Katja de Jong[2], Lalith Wijedoru[1], Masako Fukushima[3], Aniruddha Ghose[4], Rasheda Samad[4], Abdullah A. Sayeed[4], Mahtab U. Hasan[4], Md. Amir Hossain[4], Md. R. Karim[4], Stannie van den Ende[2], Thomas van der Vaart[2], Tran V. Nga[5], Pham T. Duy[5], Richard J. Maude[1], James I. Campbell[5], Stephen Baker[5], Joost W. Wiersinga[2], Tom van der Poll[2], Nicholas P. Day[1], Arjen M. Dondorp[1], Md. Abul Faiz[1], Christopher M. Parry[1]

[1]Mahidol-Oxford Tropical Medicine Research Unit (MORU), Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, [2]Center for Infection and Immunity Amsterdam (CINIMA), Academic Medical Center, Amsterdam, Netherlands, [3]Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [4]Chittagong Medical College Hospital, Chittagong, Bangladesh, [5]Wellcome Trust Major Overseas Programme, Oxford University Clinical Research Unit (OUCRU), Ho Chi Minh City, Vietnam

Typhoid fever (TF) is commonly diagnosed in febrile patients in Bangladesh but confirmatory tests are unsatisfactory. We evaluated BactAlert® blood cultures; an in-house real time PCR; and rapid antibody diagnostic tests (RDT) for TF in febrile adults and children admitted to CMCH. The RDTs were Life Assay Test-it™ Typhoid IgM lateral flow assay detecting IgM antibodies against *S. enterica* Typhi (ST) O antigen; CTKBiotech Onsite Typhoid IgG/IgM Combo Rapid-test cassette lateral flow assay detecting IgG and IgM antibodies against ST O and H antigens; and SD Bioline line assay for IgG and IgM antibodies against ST proteins. Background antibody levels were studied 40 local adult healthy controls: Life Assay RDT was positive at 1+ in 30 controls but at > 1+ in only one control; the CTK and SD Bioline kits were negative in all controls. We studied 303 febrile patients admitted to CMCH with a median (IQR) age of 13 (5-31) years and median (IQR) duration of illness before admission of 5 (2-8) days. TF was diagnosed in 57 (18.8%): 19 positive by blood culture with ST (3 also blood PCR positive); 20 blood culture negative but PCR positive in blood (15), urine (4) and faeces (2); and 18 blood culture and PCR negative but with a compatible clinical syndrome. Of the 246 patients without TF, 13 had a significant positive blood culture with other bacteria. We calculated the sensitivity, specificity, positive and negative predictive values of the three RDTs comparing those patients who had blood culture and/or PCR confirmed typhoid (n=39) with those without TF (n=246). For the Life Assay IgM LFA at a cut-off of ≥ 2+ the sensitivity, specificity, PPV and NPV were 36%, 89%, 35% and 90%; for CTK IgG/IgM assay the values were 54%, 74%, 25% and 91%; and the SD Bioline IgG/IgM assay values were 21%, 97%, 50% and 89%. The performance characteristics of the RDTs were insufficient to be clinically useful. Although the addition of PCR to BC increased the number of laboratory confirmed cases, the evaluation of RDTs is still hampered by the lack of a gold standard for TF diagnosis.

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THE FORGOTTEN SCOURGE: MODELING DYNAMICS AND CONTROL OF ENDEMIC TYPHOID IN KATHMANDU, NEPAL

Cayley Bowles[1], Virginia Pitzer[2], Bryan Grenfell[3]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Yale School of Public Health, New Haven, CT, United States, [3]Princeton University, Princeton, NJ, United States

Typhoid is a paradoxically widespread yet neglected disease. Recent estimates place the global typhoid burden from 13.5 – 26.9 million cases and 190,000 – 216,000 deaths annually, which provides a motivation to better understand typhoid dynamics. We developed an age-structured, compartmental model that was representative of the pathogen's natural history and human immune response to the infection. We fit the model to incidence data from 1997-2011 collected from Patan Hospital in Kathmandu, Nepal in order to estimate unknown model parameters. The model assumed indirect transmission was a function of rainfall and reproduced the timing of annual peaks very well, but failed to account for an upward trend in cases that began in 2000. An adjusted form of the model that incorporated antibiotic resistance reproduced both the timing and magnitude of the epidemic peaks over the entire dataset. This lends support to the hypothesis that increased use of fluoroquinolones drove the clonal expansion of the H58 haplotype, which confers nalidixic acid resistance through a mutation in DNA gyrase *gyr*A. The inclusion of migrant male workers entering Kathmandu with no previous typhoid exposure helped explain an observed shift in the age and gender distribution of cases, suggesting migration patterns partially underlie typhoid dynamics. The calibrated model estimated the basic reproductive number (R_0) to be ~4.5 in this setting. School-based vaccination was predicted to produce indirect protection and decreased typhoid incidence in the short-run, but the incidence rate is expected to rebound in about 5 years, shortly after vaccine-induced immunity wanes. As the Nepali government begins to implement school-based vaccination, government and public health authorities must recognize the limitations and potential adverse effects of a one-time vaccination campaign. Water and sanitation improvements will be critical to typhoid elimination.

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BURDEN OF LABORATORY-CONFIRMED SHIGELLOSIS INFECTIONS IN GUATEMALA 2007-2012: RESULTS FROM A POPULATION-BASED SURVEILLANCE SYSTEM

Sonia T. Hegde[1], Stephen Benoit[1], Beatriz Lopez[2], John P. McCracken[2], Chris Bernart[2], Wences Arvelo[1], Aleida Roldan[2], Cesar Rancancoj[2], Blanca Chinchilla[3], Leonard Peruski[1], Joe Bryan[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Universidad de Valle de Guatemala, Guatemala City, Guatemala, [3]Ministerio de Salud y Asistencia Social, Guatemala City, Guatemala

In Guatemala, diarrhea is the second most common cause of morbidity and mortality in children <5 years of age. The proportion of diarrheal disease caused by Shigella *sp.* remains unknown. Using data collected from the two hospitals and 10 clinics in active population-based surveillance sites in Quetzaltenango (Average High Temperature: 22°C) and Santa Rosa (Average High Temperature: 31°C) Departments, we describe the epidemiology and antimicrobial susceptibility patterns of culture-confirmed Shigella infections. Clinical, epidemiological, and laboratory data were collected on patients presenting with acute diarrhea (≥3 loose stools in 24 hours), from June 2007 - August 2012. Of 5,399 stool specimens collected from patients who met the case definition, 261 (4.8%) yielded *Shigella sp.*. Most were *S. flexneri* (59.2%) followed by *S. sonnei* (35.6%). Most (51%) infections occurred from May to August, during the rainfall season. During the 5 years, the incidence of laboratory-confirmed infections varied from 5.0 to 24.1 per 100,000 in Santa Rosa and 0.31 to 6.2 in Quetzaltenango. Most (57.9%) cases occurred in children <5 years of age; incidence in this age group were 91.9 per 100,000 in Santa Rosa and 31.1 in Quetzaltenango. Thirty (12%) patients were hospitalized, including 6 who were admitted to the intensive care unit. Three patients experienced convulsions, 5 bloody diarrhea, and 17 vomiting; there was 1 death. Over half (56%) of cases were treated with oral rehydration solution within three days of enrollment and 76% of hospitalized cases received intravenous fluids. Antimicrobial susceptibilities were tested for 260 isolates; 238 (96%) were resistant to tetracycline, 210 (83%) to trimethoprim-sulfa, and 141 (61%) to ampicillin. No isolates were resistant to the quinolone antibiotics tested. Shigella is an important cause of bacterial diarrhea in children <5 years of age in Santa Rosa and Quetzaltenango. Though limitations exist in the surveillance reporting, the reported incidence is likely an underestimate and highlight the importance of optimizing treatment regimens. Identification of specific risk factors for infection may allow for targeted prevention interventions.

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EPIDEMIOLOGY OF *STREPTOCOCCUS SUIS* INFECTION IN THE SOUTH OF VIETNAM

Nghia Ho Dang Trung[1], Constance Schultsz[2], Hoa Ngo Thi[2], Hien Tran Tinh[2], Jeremy Farrar[2]

[1]Pham Ngoc Thach University of Medicine, Ho Chi Minh City, Vietnam, [2]Centre for Tropical Medicine, Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam

Human *Streptococcus suis* infection is an emerging zoonostic disease in Southeast Asian countries. It is the most common pathogen caused bacterial meningitis at two referral hospitals for infectious diseases in Ha Noi and Ho Chi Minh City, Vietnam. To date, there has not been any information related to this pathogen in other hospitals as well as the incidence rate of this disease in Vietnam. A prospective hospital-based descriptive surveillance study was conducted from 08/2007 to 04/2010 at thirteen hospitals in central and southern Vietnam, including one district hospital, ten provincial hospitals and two central referral hospitals. Patients were recruited if they met all of the following inclusion criteria: at least one month of age; fever ≥380C (axillary); at least one of the following symptoms or signs: headache, neck stiffness, altered consciousness and focal neurological signs; and a cerebrospinal fluid (CSF) sample taken. *S. suis* was confirmed in CSF and blood samples by using classical microbiology and molecular diagnostics. A total of 1740 patients suspected central nervous system infection were recruited, in which *S. suis* was confirmed in 149 cases. *S. suis* was not found in children but it was reported as the most common pathogen of adult bacterial meningitis (149/302, 49%) in most of provincial hospitals. Overall incidence rate was 0.57/100,000 adult person-years. Incidence rate increased significantly with incremental age group. The ratio between males and females was 4:1. Pig exposures, such as breeding pigs at home, slaughtering pigs and eating raw or undercooked blood/organs, were described in 59/149 (40%) patients. Bacterial meningitis, the most common manifestation of *S. suis* infection, was responsible for 146/149 (98%) cases, and septic shock, the most serious manifestation, was reported in 3/149 cases (2%) and the overall case fatality rate (CFR) was 8% (12/149). While seasonality of *S. suis* meningitis was reported at Hue Central hospital (a tertiary hospital of the North Central of Vietnam), of which the peak month was estimated as July ($p<0.001$), it was not observed in other southern provinces ($p=0.31$). In conclusion, this study indicated that *S. suis* serotype 2 meningitis is endemic in Vietnam. Health education program on prevention should be applied to high risk groups to reduce the loss of health and economics of community.

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EPIDEMIOLOGY AND RISK FACTORS OF SEROGROUP W135 MENINGOCOCCAL DISEASE OUTBREAK IN THE GAMBIA, FEBRUARY-JUNE 2012

M. Jahangir Hossain[1], Anna Roca[1], Grant A. Mackenzie[1], Momodou Jasseh[1], Ilias Hossain[1], Shah Muhammad[1], Manjang Ahmed[1], Osuorah Donatus Chidiebere[1], Ndiaye Malick[1], Bilquees Shah Muhammad[1], Usman N. Ikumapayi[1], Baba Jeng[2], Baba Njie[2], Mamady Cham[2], Beate Kampmann[1], Tumani Corrah[1], Stephen Howie[1], Umberto D'Alessandro[1]

[1]Medical Research Council Unit, The Gambia, Banjul, Gambia, [2]Ministry of Health, Republic of The Gambia, Banjul, Gambia

In the African meningitis belt, meningococcal disease is endemic with regular outbreaks, mostly (80%) due to *Neisseria meningitidis (*Nm*)* serogroup A. In 2002-2003, a large epidemic of NmW135 occurred in this region, but not in The Gambia, where the last cases were reported in 1995. In 2012, another NmW135 epidemic occurred in the meningitis belt, including The Gambia. Between February and June 2012, the Gambian Ministry of Health and the Medical Research Council (MRC) Unit, The Gambia, investigated this outbreak in the Central (CRR) and Upper (URR) River Regions. Suspected cases were identified in Bansang Hospital, CRR, and Basse Health Centre, URR, and by visiting NmW135 cases' households. A suspected case was defined as any patient with history of acute fever and any of the following: altered consciousness, unable to feed, neck stiffness, convulsion, petechial rash or bulging anterior fontanel. Cerebrospinal fluid and blood samples were collected from hospitalized cases to identify the pathogens by culture and latex test. A confirmed case was a suspected case in which NmW135 was identified by culture and/or an antigen-specific test. A matched case-control (1:1) study was carried out. Healthy controls were matched with confirmed cases by age and village. We identified 469 suspected cases of which 114 were confirmed for NmW135. Most (65%) of them were in children <5 years old. The overall attack rate was 111/100,000 population but in children <5 years it was 5 times higher (485/100,000) than in older children and adults. The epidemic threshold (10 cases/100,000 population/week) exceeded in February and continued until April in all ages and until June in children <5 years. In the multivariate analysis, male gender (OR 1.9; 95% CI 1.0-3.7), contact with cases (OR 4.8; 95% CI 1.3-17.8), difficult breathing (OR 6.8; 95% CI 1.4-33.4) and itchy eyes (OR 4.4; 95% CI 1.3-14.4) were significantly associated with NmW135 cases. Enhanced surveillance of meningitis and multi-serogroup conjugate vaccine are recommended for the control and prevention of meningococcal epidemics.

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RODENT CONTROL PROGRAM AND LEPTOSPIROSIS PREVENTION IN SALVADOR, BRAZIL

Federico Costa[1], Jose E. Hagan[1], Guilherme S. Ribeiro[2], Renato B. Reis[1], Nivison J. Rocha[1], Maria G. Rodrigues[3], Helena M. Farias[3], Mitermayer G. Reis[1], Peter J. Diggle[4], Albert I. Ko[5]

[1]Oswaldo Cruz Foundation, Brazilian Ministry of Health, Salvador, Brazil, [2]Institute of Collective Health, Salvador, Brazil, [3]Zoonosis Control Center, Salvador, Brazil, [4]Lancaster University, Lancaster, United Kingdom, [5]Department of Epidemiology and Public Health, Yale School of Medicine, New Haven, CT, United States

Effective interventions for leptospirosis have not been identified which can be feasibly implemented in urban slum communities. Rodent Control Programs (RCP) constitute the principal strategy to prevent leptospirosis in Brazilian cities. However, RCPs are expensive and their efficacy has not been evaluated. We evaluated the efficacy of a municipal RCP to decrease rat infestation and leptospirosis incidence in Salvador (2.6 million pop.). Residences of patients with laboratory confirmed leptospirosis (years 2005-10) were geolocated and used to define 11 areas (15% of the city), containing 2,078 blocks, of equal risk for leptospirosis. During a pre-epidemic season (January-April), households from selected blocks were surveyed for rat infestation and received rodenticide application. Efficacy of the RCP was evaluated after the intervention by assessing two outcomes: 1) rat infestation by surveying 10% of treated blocks, and 2) change on incidence of leptospirosis. Kilograms of applied rodenticide and proportion of treated houses per block were used as measures of treatment intensity. These intensity proxies were used to build two mathematical models and evaluate the risk ratio of incidence of leptospirosis between pre- (2005-08) and post-intervention (2009-10) periods. A total of 671 blocks were treated in 2009 and 1,129 blocks in 2010. Surveys identified rat infestation of 25% and 26% in 2009 and 2010, respectively. 92% of the infested households in 2009 had evidence of *Rattus norvegicus*. After intervention, rat infestation decreased from 25% to 7% ($p<0.001$) in 2009 and from 26% to 15% ($p<0.001$) in 2010. The incidence of leptospirosis in the study blocks was 32.2 and 11.3 per 100,000 pop. during the pre and post-intervention periods, respectively. Using the models, the predicted reduction in incidence after maximum rodent intervention, as measured by either completeness of block coverage or kg of rodenticide, had large confidence intervals limiting our ability to evaluate the efficacy of RCP. Our study indicates that a high proportion (>25%) of households are infested with *R. norvegicus*. The RCP was able to decrease rat infestation, but because severe leptospirosis cases are rare events, it was not possible to evaluate their efficacy to decrease incidence. Further evaluations, considering more frequent events, such as mild *Leptospira* infection, may be necessary to evaluate the effectiveness of these costly interventions.

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DEVELOPMENT OF DIAGNOSTIC AIDS TO DISCRIMINATE PARTIALLY TREATED BACTERIAL MENINGITIS (PTBM) FROM VIRAL MENINGITIS/ENCEPHALITIS (VM/EN)

Hong Chau T. Tran[1], Marcel Wolber[2], Tan Le[2], Hoang Mai Nguyen[2], Thanh Hang Hoang[3], Nghia Ho Dang Trung[3], Rogier Van Doorn[1], Jeremy Farrar[4]

[1]Hospital for Tropical Diseases, Ho Chi Minh, Vietnam, [2]Centre for Tropical Medicine,Oxford University Clinical Research Unit, Ho Chi Minh, Vietnam, [3]Pham Ngoc Thach University of Medicine, Ho Chi Minh, Vietnam, [4]Centre for Tropical Medicine, Oxford University Clinical Research Unit, Ho Chi Minh, Vietnam

The diagnosis of ptBM is difficult. Discrimination of cases from those of VM/EN by clinical features alone is often impossible. We aimed to create a simple diagnostic aid for ptBM in adults on the basic laboratory features. We compared the laboratory features on admission of 374 adults at HTD, Vietnam who satisfied diagnostic criteria for ptBM (n=291) or VM/EN (n=83). Laboratory features independently predictive of ptBM were modelled by logistic regression according to Bayesian information criterion (BIC) and by classification- tree (C-T) method. Prognostic accuracy was summarized by sensitivity/ specificity / positive predictive value (PPV)/ negative predictive value (NPV). To assess potential over fitting of our models, all performance measures were bootstrap corrected for optimism. BIC defined three characteristics independently predictive of a diagnosis of ptBM from VM/EN: cerebrospinal fluid (CSF) neutrophil proportion (N%), CSF: blood glucose, and log2 (CSF lactate). Using these three predictors we developed a diagnostic nomogram. Our C-T constructed on two predictors (CSF lactate and CSF white cell count) which is more simple than nomogram but less sensitivity, specificity, PPV and NPV than those in BIC (0.979, 0.923, 0.978 and 0.929, and 0.984, 0.962, 0.988 and 0.950, respectivively). This study suggests that simple laboratory data can help in the diagnosis of adults with ptBM, particularly in setting with limited microbiological resources.

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VACCINATION WITH A GENETICALLY MODIFIED FILARIAL CYSTEINE PROTEASE INHIBITOR-2 PROTECTS GERBILS AGAINST *BRUGIA MALAYI* AND MICE AGAINST *ONCHOCERCA VOLVULUS* INFECTION

Sridhar Arumugam[1], Bin Zhan[2], David Abraham[3], Jessica Hess[3], Simon A. Babayan[4], Judith E. Allen[5], David W. Taylor[5], Danielle Ward[1], Thomas R. Klei[1], Sara Lustigman[6]

[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]Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow and Moredun Research Institute, Glasgow, United Kingdom, [5]University of Edinburgh, Edinburgh, United Kingdom, [6]Lindsley F. Kimball Research Institute, New York Blood Center, New York, NY, United States

Cysteine protease inhibitors or cystatins are reversible, tightly binding inhibitors of cysteine proteases. Filarial cysteine protease inhibitors have been ascribed to participate in worm's development as well as to contain immunomodulatory properties. They are hypothesized to play an important role in the establishment of infection by suppressing host immune responses, and therefore are good candidates for vaccine development. Expressed recombinant wild-type *B. malayi* cysteine protease inhibitor-2 (Bm-CPI-2) and *Onchocerca volvulus* cysteine protease inhibitor-2 (Ov-CPI-2) in *E. coli* showed strong inhibitory activity against Cathepsin L. Since the wild-type cystatin is a strong immune suppressor and therefore could inhibit host immune response upon immunization, the amino acid Asn at position 66 related to its asparaginyl endopeptidase inhibition activity was mutated to Lys66 in order to inactivate its immune suppressive activity and therefore enhance its protective immunity. DNAs encoding the Bm-CPI-2 or Ov-CPI-2 minus the signal peptides, with Asn66 mutated to Lys66 (Bm-CPI-2M, Ov-CPI-2M) were synthesized by GenScript and subsequently subcloned and expressed in the *E. coli* expression vector pET41a. Mongolian gerbils were immunized with 25 µg of the recombinant Bm-CPI-2M intraperitoneally with alum as the adjuvant three times, two weeks apart. The gerbils were challenged with infective L3 larvae subcutaneously and the parasites were recovered on day 42 post-infection. Vaccination with Bm-CPI-2M resulted in 48% reduction in worm burden in comparison to the Alum control group. Measurement of Bm-CPI-2M specific IgG by ELISA showed elevated levels of specific antibody response in the Bm-CPI-2M vaccinated gerbils. Vaccination of mice with the *O. volvulus* modified cystatin, Ov-CPI-2M, in alum also induced protection against larvae implanted subcutaneously within diffusion chambers, resulting in a 27% reduction in parasite survival. The immunized mice developed antigen-specific IgG responses. Our results confirm the CPI-2M vaccine-mediated protection obtained in the murine model of filariasis Litomosoides sigmodontis (Babayan et al. 2012), and extend it to filarial parasites of humans. In conclusion, the genetically modified filarial cysteine protease inhibitor-2 is a promising candidate for use in prophylactic vaccines against filariasis.

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EARLY DNA METHYLATION EVENTS IN UROGENITAL SCHISTOSOMIASIS AND THEIR IMPLICATIONS FOR INFLAMMATION-INDUCED BLADDER CANCER

Simon Conti, Jared Honeycutt, **Michael Hsieh**

Stanford University School of Medicine, Stanford, CA, United States

Urogenital schistosomiasis is linked to inflammation-associated bladder cancer. Inflammation-induced DNA methylation of tumor suppressor genes has been implicated in various forms of carcinogenesis. We hypothesized that some of these DNA methylation changes could be detected early after induction of experimental urogenital schistosomiasis. We combined our established mouse model of urogenital schistosomiasis with reduced representation bisulfite sequencing (genome-wide methylation analysis). Mice underwent sub-epithelial bladder injections of *Schistosoma haematobium* eggs or vehicle. Other mice received drinking water containing nitrosamine, an established urothelial carcinogen. After two weeks of exposure mice were sacrificed and their urothelia dissected from the detrusor and granuloma. DNA was extracted from each specimen and the restriction enzyme Msp1 used to cleave CpG islands. After bisulfite treatment samples were purified to a length of 175-225 bp and amplified by PCR. Next generation sequencing was performed with the Illumina Hi-Seq platform. The output was aligned with the UCSC M. Musculus genome v10 using Bismark software. Methylation analysis was performed with Methylkit and IGV. Egg-injected mice featured major alterations in their methylome (vs control mice) after two weeks of treatment. Bases with a depth of sequencing of less than 10 were excluded from analysis, and differential methylation was defined as a different of greater than 25% with a p-value of less than 0.05. 13,333 cytosines were hypermethylated and 6,244 were hypomethylated. Of these differentially methylated bases 1019 were found to be within 1000 base pairs of a transcription start site for a known gene. Six of these genes are part of the Wnt canonical pathway, which is related to cell proliferation. A CpG upstream of the Wnt Inhibitory Factor-1 gene, a gene silenced by hypermethylation in bladder tumors and other cancers, was methylated 54% of the time in egg-injected mice, 34% in nitrosamine-fed mice and 7% in control mice. This methylation event, along with our profiling of the DNA methylome of mice with experimental urogenital schistosomiasis, is the first of its kind, and may lead to an understanding of the sentinel events of urogenital schistosomiasis-associated bladder carcinogenesis.

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INFECTION WITH CARCINOGENIC LIVER FLUKE *OPISTHORCHIS VIVERRINI* MODIFIES INTESTINAL AND BILIARY MICROBIOME

Jordan L. Plieskatt[1], Raksawan Deenonpoe[2], Jason P. Mulvenna[3], Lutz Krause[3], Banchob Sripa[2], Jeffrey M. Bethony[1], **Paul J. Brindley**[1]

[1]The George Washington University, School of Medicine and Health Sciences, Department of Microbiology, Immunology and Tropical Medicine, Washington, DC, United States, [2]Tropical Disease Research Laboratory, Department of Pathology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand, [3]Queensland Institute of Medical Research, Brisbane, Australia

Opisthorchis viverrini is a fish-borne trematode endemic in East Asia. Following ingestion, the flukes locate to the biliary tract, where chronic infection frequently leads to cholangiocarcinoma (CCA). The precise mechanism(s) by which *O. viverrini* infection culminates in CCA is not known. One unexplored aspect is its influence on the host microbiome. In the Syrian hamster, infection with this pathogen reliably leads to CCA. Genomic DNAs of microbiota from colorectal contents and bile of hamsters and *O. viverrini* were examined in this model of fluke-induced CCA. Sequences of regions 7, 8 and 9 of prokaryotic 16S rRNA genes were amplified, pyrosequenced, operational taxonomy units classified, and analysis of community diversity undertaken. Of ~1,000,000 sequences, 536,009 could be assigned to 20 phyla and 273 genera of bacteria or Archaea. Diversity analyses revealed that fluke infection perturbed the gastrointestinal tract microbiome, increasing Lachnospiraceae, Ruminococcaceae and Lactobacillaceae while decreasing Porphyromonadaceae, Erysipelotrichaceae and Eubacteriaceae ($p \leq 0.05$). In addition, >60 prokaryote species were detected in the biliary system, which confirmed bacteriobilia and a remarkable community associated with the parasites. These fluke-associated microorganisms included potential pathogens from the *Enterobacteriaceae* and Listeriaceae and others from external environments including cyanobacteria and Deinococci. Given that opisthorchiasis is distinguished from other helminth infections by a robust inflammatory phenotype, with conspicuously elevated interleukin 6, and that inflammation of the biliary system leads to periductal fibrosis that is a precursor to CCA, the flukes as well as their microbiota might together drive this distinctive immune response.

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IMMUNOMICS-BASED IDENTIFICATION OF SCHISTOSOMIASIS VACCINE ANTIGENS: AN INTEGRATED DISCOVERY AND VALIDATION APPROACH

Mark S. Pearson[1], Soraya Gaze[1], Patrick Driguez[2], Tiago Mendez[3], Denise Doolan[2], Angela Trieu[2], Don McManus[2], Fernanda Cardoso[2], Rie Sasaki[4], Philip Felgner[4], Jeffrey Bethony[5], Alex Loukas[1]

[1]James Cook University, Cairns, Queensland, Australia, [2]Queensland Institute of Medical Research, Brisbane, Queensland, Australia, [3]Federal University of Minas Gerais, Belo Horizonte, Brazil, [4]University of California, Irvine, CA, United States, [5]George Washington University, Washington, DC, United States

Schistosomiasis is a neglected tropical disease affecting >230 million people and causes over 200,000 deaths annually. To identify new vaccine antigens and assess their potential protective efficacy and safety, we used an immunomics approach with sera from putatively resistant (PR) and chronically infected (CI; stratified by infection intensity) people in a high transmission area for schistosomiasis in Brazil. We selected mostly tegumental *Schistosoma mansoni* and *S. japonicum* proteins, produced them using an *in vitro* rapid translation system (RTS) and printed them to generate the first protein microarray for a multi-cellular pathogen. Arrays were screened to detect IgG subclass and IgE responses; antigens which showed preferential/unique recognition by IgG1/3 from PR individuals were up-selected, and those that were the target of potentially deleterious IgE responses (in terms of vaccine-induced hypersensitivity) were down-selected. We detected strong correlations between the number of antigens recognized and infection intensity for all antibody subclasses, most notably IgE. Surprisingly, PR individuals produced little IgE but instead made robust IgG1/3 responses to a small number of antigens exposed on the parasite surface, highlighting their potential as vaccine antigens. Cluster analysis was performed to identify antigen clusters based on their antibody recognition profiles. Two clusters contained antigens that were preferentially recognized by IgG1/3 of PR individuals but were not major targets of IgE; these clusters included the previously described vaccine candidates Sm-TSP-2 and Sm29, as well as a panel of new antigens that have not been previously described. We have shown here the use of a high throughput immunomics approach to profile antibody responses from PR and CI individuals that has unearthed a suite of novel potentially protective and safe schistosomiasis vaccine antigens. To complement our human subjects-oriented antigen discovery approach, we are also probing arrays with sera from non-human primates that have been vaccinated with irradiated schistosome cercariae.

1491

ELEVATED ARGINASE 1 AND LOW NITRIC OXIDE SYNTHASE 2 PBMC EXPRESSION: EVIDENCE OF ALTERNATIVE MACROPHAGE ACTIVATION IN CHILDREN WITH MALARIA

Florence Savatory

Hubert Kairuki University, Dar es salaam, United Republic of Tanzania

We demonstrated earlier that malaria infection is associated with low serum arginine levels and low expression of nitric oxide synthase2 (NOS2) leading to diminished nitric oxide (NO) production and endothelial dysfunction. We established that NO is protective in malaria. The mechanism of diminished NO production is not well understood, but it is likely to be multi¬factorial. Increased metabolism of arginine by arginases and suppression of NOS2 expression likely play roles. Alternative macrophage activation (M2) is initiated by Th2 cytokines such as IL-4, IL-10, and IL-13. M2 activation is also associated with increased arginase1, decreased NOS2 and NO expression by monocytes-macrophages, and likely less resistance to malaria infection and/or malaria disease. The aim of this study was to investigate the markers for monocytes/macrophage in PBMCs from children *Plasmodium falciparum* infection. Children aged 6mo to 9 yr were recruited from Amana and Mwananyamala hospitals in Dar es Salaam, Tanzania, and categorized (modified WHO criteria) as severe malaria (SM), uncomplicated malaria (UM),or healthy control (HC). We prospectively measured PBMC mRNA for arginases 1 and 2, and NOS2 using quantitative RT-PCR.Results were analyzed using Prism 5 software and Mann-Whitney non-parametric comparison analysis. We enrolled 80 SM, 80 UM, and 48 HC participants. There was marked increase in PBMC arginase 1 mRNA in children with malaria compared to healthy controls (4.5 fold in UM and 9.3 fold in SM; p = 0.02 and 0.008 respectively), while NOS2 mRNA was lower in SM and UM than in HC (p = 0.0001) for each comparison. PBMC arginase 2 mRNA was lower in SM compared to HC, but it was not statistically significant (p = 0.89). In conclusion, malaria infection in children is associated with increased arginase 1, and decreased NOS2 and arginase 2 mRNA expressions. This is characteristic of alternative macrophage activation and may partly explain the low serum arginine and diminished NO production in malaria. Assessment of arginase activity in PBMCs (and purified mononuclear phagocytes) during malaria infection is warranted to fully establish the role of alternatively activated monocytes-macrophages in the hypoarginaemia observed during the malaria infection.

1492

IMBALANCE OF INFLAMMATORY AND ANGIOGENIC FACTORS IN EARLY PREGNANCY ARE ASSOCIATED WITH PRETERM BIRTH IN A PROSPECTIVE COHORT OF MALARIA-EXPOSED TANZANIAN WOMEN

Chloe E. McDonald[1], Anne Marie Darling[2], Andrea L. Conroy[1], Kyla Hayford[1], Nimerta Rajwans[1], Said Aboud[3], Willy Urassa[3], Wafaie Fawzi[2], Kevin C. Kain[1]

[1]University of Toronto, Toronto, ON, Canada, [2]Harvard University, Boston, MA, United States, [3]Muhimbili University of Health and Allied Sciences, Dar es Salaam, United Republic of Tanzania

Malaria in pregnancy is associated with several adverse birth outcomes including preterm birth (PTB). PTB is now the leading cause of perinatal mortality globally, however there are currently no diagnostic tools to predict pregnancies at risk of PTB. Based on the hypothesis that altered placental angiogenesis and inflammation early in pregnancy lead to PTB, we examined if levels of inflammatory and angiogenic mediators, measured early in pregnancy (< 27 weeks gestation), were predictive of PTB in a cohort of women living in a region of high malaria transmission. Plasma samples were collected from a prospective cohort of 432 primigravid women at enrollment (12-27 weeks gestation). A total 63 women subsequently delivered preterm (< 37 weeks gestation). Levels of 18 biomarkers reflective of angiogenic and/or inflammatory pathways (Ang-1, Ang-2, Ang-L3, VEGF, sFLT-1, sTNFR2, PlGF, MIP-1β, MCP-1, Leptin, IL-1β, IL-18 BP, sICAM-1, FAC-D, sEndoglin, CRP, CHI3L1, C5a) were analyzed by ELISA. Plasma levels of PlGF (*P*=0.04), IL-18 BP (*P*=0.002), sICAM-1 (*P*=0.03), sEndoglin (*P*=0.0005), CHI3L1 (*P*=0.002), sTNFR2 (*P*=0.05) were higher at enrollment in women who subsequently experienced PTB compared to women who delivered at term. Based on multiple analytic methods, plasma levels of IL-18BP, CHI3L1 and sEndoglin were elevated at enrollment, in women who went on to deliver preterm. Combinatorial strategies were applied in an attempt to improve predictive accuracy. Combining biomarker data (sICAM-1 and CHI3L1) with clinical and demographic data improved our predictive model of PTB over that possible with clinical data alone (*P*=0.0002). In conclusion, in this cohort of Tanzanian women, levels of angiogenic and inflammatory mediators measured early in pregnancy, were associated with subsequent PTB. These proteins provide insight into the underlying mechanism of PTB and may have clinical utility as early biomarkers of preterm delivery. Given the high rates of PTB in malaria endemic regions, there is a critical need to develop early diagnostic tools to identify pregnancies at risk of PTB.

1493

PLACENTAL MALARIA INDUCES EXCESSIVE VASCULOGENESIS

Tara C. Bracken[1], Samantha Burton[1], Ashley McMichael[1], Carlos Abramowsky[2], Simon Owino[1], Julie Moore[1]

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

Placental malaria (PM) results from sequestration of *Plasmdium falciparum*-infected erythrocytes and the resulting inflammatory responses in the maternal placental blood space. PM induces maternal anemia, preterm birth, low birth weight, or stillbirth, especially in primigravidae. PM may also promote local hypoperfusion or hypoxia, inducing neovasculogenesis in the fetal placental compartment. Excessive vasculogenesis can result in chorangiosis (CHOR), defined as at least 10 vascular channels (VC) in at least 10 terminal villi in 10 low power microscopic fields in three discreet regions of the placenta. CHOR is rare but is enhanced in preeclampsia and diabetes and is associated with neonatal morbidity and mortality. To determine the extent to which PM induces CHOR, placentae were collected from 18 consenting primigravidae at two public hospitals in Kisumu, Kenya. Malaria status and PM chronicity were estimated by microscopic examination of placental blood smears and PCR and by placental histology. Patients were separated into the following groups: uninfected (UN, n=2), active/active chronic (A/AC, n=9) infection, and past/past chronic infection (P/PC, n=7). Thin sections from three fixed placental tissue sections were hematoxylin and eosin-stained, and thirty micrographic images at 200X magnification (to approximate ten low power fields at 100x) were captured from each section. Number of villi and VC therein were counted. Whereas UN and P/PC placentae were equivalent, A/AC placentae had statistically significantly higher median numbers of VC/villus (*P*<0.0001). Moreover, the latter group also had a significantly higher percentage of villi with ten or more VC relative to the other two groups (27.2±1.6% versus 20.5±0.8% (UN) and 20.7±2.0% (P/PC), *P*=0.0355). Further analysis of these samples is underway to evaluate whether these samples meet the clinical definition of CHOR and associations with birth outcomes. In addition, assessment of markers of angiogenesis promises to provide insight into the molecular mechanisms underlying this phenomenon in PM.

1494

RETINAL MICROVASCULAR DYSFUNCTION IN PEDIATRIC CEREBRAL MALARIA IS ASSOCIATED WITH DEATH AND NEUROLOGICAL SEQUELAE

Ian J. MacCormick[1], Simon J. Glover[2], Nicholas A. Beare[3], Gabriela Czanner[3], Macpherson Mallewa[1], Terrie T. Taylor[4], Malcolm E. Molyneux[1], Simon P. Harding[3]

[1]Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Queen Elizabeth Central Hospital, College of Medicine, Blantyre, Malawi, [2]Department Ophthalmology, Addenbrooke's Hospital, Cambridge, United Kingdom, [3]Department of Eye and Vision Science, University of Liverpool, Liverpool, United Kingdom, [4]Blantyre Malaria Project, University of Malawi College of Medicine, Blantyre, Malawi

Malarial retinopathy (MR) appears to reflect brain pathogenesis in pediatric cerebral malaria (CM), since it is related to mortality and highly predictive of brain histopathology. MR is associated with abnormal fluorescein angiography (FA), but mortality associated with these abnormalities is unknown. We aimed to characterize FA abnormalities and their relationship to clinical outcomes. Two ophthalmologists graded admission angiograms in 170 consecutive patients with pediatric CM from 2006 to 2010 (WHO criteria, including retinopathy negative cases). Variation between eyes was assessed using Cohen's kappa statistic. Associations between FA abnormalities, mortality, and presence of neuro-disability on discharge were assessed using left eye data and Fisher's exact test. In our series 118 survived, 25 survived with neuro-disability, and 27 died. All FA signs were consistent between right and left eyes. Frequencies of features were: Capillary non-perfusion (CNP), macular 82%, peripheral 84%; Intravascular filling defect (by vessel type): large 38%, small 14%, occluded 22%; Vessel leak: small macular 56%, small peripheral 49%, mid/large 16%, disc 52%. Severity of macular CNP (p=0.02) and presence of peripheral small vessel leakage (p=0.03) were significantly associated with death and neurological disability; peripheral CNP (p=0.45), and macular small vessel leakage were not (p=0.09). This is the largest analysis of retinal angiography in pediatric CM to date. Retinal CNP is very common. Severity of macular CNP, and the presence of fluorescein leakage from small peripheral retinal vessels are associated with death and neuro-disability. CNP indicates ischemia and matches areas of retinal whitening seen clinically. This result is consistent with a known association between macular whitening and death. FA leakage results from breakdown of the blood-retinal barrier, which is similar to the blood-brain barrier. Our results suggest that central nervous system ischemia and leakage across blood-tissue barriers may be important contributors to the severity of pediatric CM.

1495

FATAL PEDIATRIC CEREBRAL MALARIA IS ASSOCIATED WITH INTRAVASCULAR INFLAMMATION AND COAGULATION THAT IS EXACERBATED BY HIV-1 CO-INFECTION

Sarah Hochman[1], Theresa Madaline[2], Karl Seydel[3], Terrie Taylor[3], Danny Milner[4], Sunhee Lee[1], Kami Kim[1]

[1]Albert Einstein College of Medicine of Yeshiva University, Bronx, NY, United States, [2]Montefiore Medical Center, Bronx, NY, United States, [3]Michigan State University, East Lansing, MI, United States, [4]The Brigham and Women's Hospital, Boston, MA, United States

Most malaria deaths occur in sub-Saharan African children and are due to various severe malaria syndromes, including cerebral malaria (CM). In Malawi, the overall prevalence of HIV-1 is 10%, with lower seroprevalence in children. The entire population is at risk for malaria. High rates of malaria/HIV co-infection are likely but effects of HIV on CM pathogenesis and outcome are unknown. The Blantyre Malaria Project (BMP) has found 3 patterns of brain pathology in children who met clinical criteria for CM: sequestration alone (CM1), sequestration plus intra- and peri-vascular pathology (CM2) and no sequestration (CM3). In the BMP cohort, the HIV+ rate is 13% overall and 20% in autopsied patients. 60% of autopsies with the CM1 pattern are HIV+ compared to 18% with CM2 and 6% with CM3. To determine whether HIV co-infection affects the pathophysiology of CM, we performed immunohistochemistry on brain tissue from autopsied patients with clinically-defined CM. We examined 10 cases with the CM1 pattern, 10 with CM2 and 10 with CM3 or coma of other cause (COC). Five from each group were HIV+. Brain sections were labeled for HIV-1 p24, ionized calcium binding adapter molecule 1 (Iba1), a marker for microglia and monocytes, and CD61, a platelet marker. No HIV-1 p24 was seen. We observed intravascular Iba1+ monocytes containing hemozoin that completely filled small vessels and adhered to the walls of larger vessels, accompanied by platelet clumps. This was significantly increased in CM1/2 cases compared with CM3/COC cases and was significantly increased in HIV+ CM1/2 cases compared to HIV- CM1/2 cases. Most HIV+ CM1/2 cases had mild immunosuppression by WHO HIV clinical staging and the total lymphocyte counts of HIV+ CM1/2 cases were similar to those of HIV- CM1/2 cases. HIV+ CM1/2 cases were significantly older than HIV- CM1/2 cases. We hypothesize that the intravascular inflammation and coagulation seen in CM autopsies contribute to the pathogenesis of pediatric CM and that dysregulation of these processes in HIV infection contribute to CM mortality.

1496

MALARIA PIGMENT (HEMOZOIN) AND EXTRAVASATED FIBRINOGEN ARE ASSOCIATED WITH RETINAL VESSEL LEAKAGE AND HEMORRHAGES IN MALAWIAN CHILDREN WITH CEREBRAL MALARIA

Valentina Barrera[1], Paul S. Hiscott[1], Nick A. Beare[2], Alister G. Craig[3], Valerie A. White[4], Ian J. MacCormick[5], Simon J. Glover[6], Terrie E. Taylor[7], Malcolm E. Molyneux[5], Simon P. Harding[1]

[1]Department of Eye and Vision Science, University of Liverpool, Liverpool, United Kingdom, [2]St. Paul's Eye Unit, Royal Liverpool University Hospital, Liverpool, United Kingdom, [3]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [4]Department of Pathology and Laboratory Medicine and Ophthalmology and Visual Sciences, University of British Columbia, Vancouver, BC, Canada, [5]Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Queen Elizabeth Central Hospital, College of Medicine, Blantyre, Malawi, [6]Department Ophthalmology, Addenbrooke's Hospital, Cambridge, United Kingdom, [7]Blantyre Malaria Project, University of Malawi College of Medicine, Blantyre, Malawi

Malarial retinopathy (MR) distinguishes cerebral malaria (CM) from non-malarial causes of coma. White-centered retinal hemorrhages are a common clinical feature and vessel leakage due to blood-retina barrier breakdown is found angiographically. We conducted a *post mortem* clinicopathological study to localize features of microvascular pathology affecting neural retina in Malawian children. Histopathological analyses were carried out on 7 cases: 5 cases with clinically defined CM during life showed MR features, and 2 patients with non-malaria comas had no evidence of MR. Retinal microvascular pathology was assessed by presence of: i) extraerythrocytic hemozoin (HZ) in retinal capillaries and venules, on the basis of hematoxylin-eosin staining (H&E); ii) perivascular leakage, with anti-fibrinogen (FGN) and anti-albumin immunohistochemistry (IHC); iii) retinal hemorrhages, with H&E and specific IHC markers (CD45 for inflammatory cells; collagen, smooth muscle actin, CD34 for vessels remnants). The MR cases were classified in two groups: one case had 16% vessels with HZ (Group 1), and four cases showed HZ in a median of 49% (min-max 25-82%) vessels (Group 2). Group 1 showed patchy focal leakage only in the retinal venules with HZ, and no hemorrhages. Each case in Group 2 presented ≥ 5 retinal hemorrhages, characterized by a white-center of FGN which accumulated in the perivascular space together with HZ and inflammatory cells. In the non-MR controls HZ was absent, and one case had retinal hemorrhages secondary to head injury and intracranial hemorrhage (Terson syndrome). HZ was found associated with features of retinal vascular pathology in severe MR cases, concurring with evidence of CM vascular pathology in the brain such as ring hemorrhages. Extravasated FGN from venules with HZ, as well as its presence in the center of retinal hemorrhages, suggest leakage can evolve with disruption

of retinal layers. Further studies on a potential temporal link between the two features can help us to define consequences of blood retinal barrier breakdown in MR.

1497

NON-INVASIVE PULSE OXIMETRY TO PREDICT MORTALITY IN AFRICAN CHILDREN WITH MALARIA

Andrea L. Conroy[1], Michael Hawkes[1], Chandy C. John[2], Sophie Namasopo[3], Robert O. Opoka[4], Kevin C. Kain[1]

[1]University of Toronto, Toronto, ON, Canada, [2]University of Minnesota, Minneapolis, MN, United States, [3]Jinja Regional Referral Hospital, Jinja, Uganda, [4]Makerere University, Kampala, Uganda

Between February 2012 and April 2013 we enrolled 1677 children in a prospective observational study of children 2 months to 5 years admitted to Jinja Regional Referral hospital with a history of fever or an axillary temperature >37.5°C and known disease outcomes. The mean age of children enrolled was 1.65 years old, and malaria was the most frequent reason for admission with 76% of children having a diagnosis of malaria based on microscopy and/or positive 3-band RDT (pLDH/HRP2). The mortality rate for children admitted with malaria was 3.1%. We evaluated whether non-invasive pulse oximetry would predict disease outcome in malaria and compared the findings to venous lactate, an established prognostic marker in malaria. We used receiver operator characteristic (ROC) curves to assess the predictive ability of the biomarkers. The area under the curve (AUC) for the oxygen saturation (Sp02) was 0.69 (95% CI, 0.59-0.80; p<0.0001), and a Sp02 less than 92% was 97% sensitive and 37% specific in predicting mortality. In addition to Sp02, the Masimo Pulse CO-oximeter has the capacity to measure the perfusion index (PI), which is the ratio of pulsatile blood flow to non-pulsatile static blood flow in peripheral tissue. The PI is a more sensitive and objective measure of peripheral perfusion than measuring capillary refill. The PI measured on children's finger tips or toes had an AUC of 0.68 (0.57-0.78, p<0.0001), and a PI less than 0.20 was 98% sensitive and 17% specific in predicting mortality. Initiation of appropriate life-saving measures (oxygen administration, treatment for shock) in children with low Sp02 or a low PI resulted in marked patient improvement. Venous lactate ≥5.5mmol/L had an AUC of 0.80 (09% CI, 0.71-0.90; p<0.0001) and a sensitivity and specificity of 81% and 77%. These data suggest that pulse oximetry alongside assessment of venous lactate may be useful in the triage and treatment of children with severe malaria. Additional advantages in pulse oximetry are low operating costs and real-time patient monitoring.

1498

A NOVEL, KINETOPLASTID-SPECIFIC CAMP SIGNALING PATHWAY - A PROMISING DRUG TARGET

Sabine Bachmaier[1], Matthew K. Gould[2], Juma A. Ali[2], Sam Alsford[3], Jane Munday[2], David Horn[3], Harry P. de Koning[2], Michael Boshart[1]

[1]University of Munich (LMU), Biocenter, Section Genetics, Martinsried, Germany, [2]Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom, [3]Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, United Kingdom

The signaling molecule cAMP plays crucial regulatory roles in almost all eukaryotic cells. In *T.brucei*, the genetic or pharmacological manipulation of the intracellular cAMP concentration results in severe cytokinesis phenotypes with subsequent cell death. Consequently, the cAMP-specific phosphodiesterases have been validated as excellent drug targets. However, the *T. brucei* orthologue of the major downstream target of cAMP, the cAMP-dependent protein kinase (PKA), is not activated by cAMP, nor have homologues of other known mammalian cAMP effectors been identified. We thus used genome-wide RNAi library screening to select cells resistant to Cpd A, a novel and highly specific PDE inhibitor, which kills bloodstream trypanosomes via elevated intracellular cAMP. Four candidate genes (CARP1-4: cyclic AMP response proteins) were identified, whose depletion confers different degrees of resistance to Cpd A. CARP1, a protein unique to kinetoplastid parasites has two predicted cNMP binding domains, and its depletion resulted in up to 200-fold Cpd A resistance. We suggest that this protein is a primary cAMP sensor and CARP2-4 proteins may be components of a novel cAMP signaling pathway. Binding of cAMP to CARP1, physical and genetic interactions among the CARP proteins, and their subcellular localisation are under investigation. We propose the novel kinetoplastid-specific cAMP signaling cascade as promising new drug target for Human African Trypanosomiasis and possibly other kinetoplastid diseases.

1499

CHARACTERIZATION OF THE SMALL PROTEOME OF *TRYPANOSOMA BRUCEI*

Megan Ericson[1], Michael Janes[1], Falk Butter[2], Samson Obado[3], Michael Rout[3], Matthias Mann[2], Elisabetta Ullu[1], Christian Tschudi1

[1]Yale University, New Haven, CT, United States, [2]Max Planck Institute for Biochemistry, Martinsried, Germany, [3]Rockefeller University, New York, NY, United States

Advances in genomics research are providing new avenues to a more holistic understanding of pathogens. An RNA-Seq transcriptome study from our lab identified 1,114 novel transcripts in *Trypanosoma brucei* of which 993 have at least one potential ORF. The majority fit into the category of short ORFs (sORFs), since the predicted protein is between 25 and 100 amino acids in size. Mining mass spectrometry data sets revealed 42 novel transcripts that encode a sORF matching to at least one unique peptide, suggesting that these proteins are expressed. Thus, the trypanosome proteome appears larger than previously believed. To begin to address the possible function of small proteins in *T. brucei*, all 42 novel transcripts were down-regulated by RNAi and 7 were determined to be essential in procyclic trypanosomes. Each lethal phenotype was rescued by co-expressing an RNAi-resistant construct, further validating the significance of these small proteins. The 7 essential sORFs are only found in trypanosomatids: five are widespread, while two are specific to African trypanosomes. For example, the essential protein encoded by Tb10.NT87 is 64 amino acids long and localizes to the matrix of the mitochondria, as shown by immuno EM, and a karyopherin-like protein has been identified as a potential interacting partner. On the other hand, Tb11.NT29 encodes 62 amino acids with a predicted trans-membrane domain and is localized on the surface of procyclic- and bloodstream-form trypanosomes. In addition, essential small proteins localize to the nucleolus, cytoplasm, and a perinuclear compartment of the cell, highlighting the diverse biological roles they are likely to play. Experiments are in progress to assess the essentiality in bloodstream form trypanosomes and to identify interacting partners.

1500

FUNCTIONAL VALIDATION OF HOST METABOLIC PATHWAYS AS CRITICAL REGULATORS OF *TRYPANOSOMA CRUZI* AMASTIGOTE GROWTH IN HUMAN CARDIOMYOCYTES

Kacey Caradonna, Sheena Shah-Simpson, Barbara Burleigh

Harvard School of Public Health, Boston, MA, United States

The intracellular amastigote stage of Trypanosoma cruzi is a critical target for vaccine and drug development for the prevention and treatment of human Chagas' disease - the leading cause of infectious cardiomyopathy. Despite the importance of amastigotes in infection and disease, we have a limited understanding of host factors that contribute to the growth and survival of these parasites. In a recent genome-wide RNA interference (RNAi) screen, host metabolic networks centered around energy production, nucleotide metabolism, pteridine biosynthesis, and fatty acid oxidation were identified as key processes that support T. cruzi infection in HeLa cells (Caradonna et al. 2013. Cell Host & Microbe 13

108-117). As a more relevant in vitro infection model, we are exploiting human induced pluripotent stem cell (iPSC)-derived cardiomyocytes for functional validation studies. iPSC- cardiomyocytes are transcriptionally and electrophysiologically similar to adult cardiomyocytes and amenable to high-throughput RNAi screening applications. Using this system, a number of 'hits' that originally surfaced in our RNAi screen have now been validated in cardiomyocytes, including pyruvate dehydrogenase kinase 4 (PDK4). PDK4 regulates the fuel utilization balance in mammalian cells where depletion results in reduced fatty acid oxidation and reduced parasite growth. Coupling host gene knockdown studies with sensitive extracellular flux measurements in live cardiomyocytes has allowed us to confirm the metabolic phenotypes associated with targeted host gene knockdown. We are currently exploiting this experimental system to elucidate the contribution of host lipid metabolism to T. cruzi amastigote growth and survival. The primary objective of this study is to gain mechanistic insight into the relationship between host metabolism and T. cruzi amastigote growth. This knowledge is fundamental to a broader understanding of intracellular parasitism and will open the door to potential alternative interventions.

1501

ROLE OF TLR9 SIGNALING IN EXPERIMENTAL *LEISHMANIA BRAZILIENSIS* INFECTION

Tiffany Weinkopff[1], Anita Mariotto[2], Gregoire Simon[2], Yazmin Hauyon-La Torre[2], Floriane Auderset[2], Steffen Schuster[2], Haroun Zangger[3], Nicolas Fasel[3], Aldina Barral[4], Fabienne Tacchini-Cottier[2]

[1]Department of Biochemistry, World Health Organization-Immunology Research and Training Center, University of Lausanne, Epalinges, Switzerland, [2]Department of Biochemistry, University of Lausanne, Epalinges, Switzerland, [3]Centro de Pesquisas Goncalo Moniz, Fundacao Oswaldo Cruz, (FIOCRUZ) and Faculdade de Medicina, Universidade Federal da Bahia, Salvador, Brazil

Infection with *Leishmania braziliensis* causes cutaneous or mucocutaneous leismaniasis in humans. TLR9 expression has been found in granulomas of lesions in *L. braziliensis*-infected individuals. *L. braziliensis* inoculation in mice induces very small lesions that are self-healing whereas deficiency in the TLR adaptor molecule, MyD88, render mice susceptible to infection. The TLR receptor involved has not been identified, prompting us to investigate if TLR9 triggering by the parasite contributes to the strong resistance to infection observed in *L. braziliensis*-inoculated mice. The parasites activated wild-type (WT) dendritic cells (DCs) in vitro, but not DCs derived from TLR9-/- mice. TLR9-/- mice inoculated with *L. braziliensis* exhibited a transient susceptibility characterized by increased lesion size and parasite burden compared to WT mice. Surprisingly, elevated levels of IFNγ were measured at the site of infection and in draining lymph node T cells of TLR9-/- mice at the peak of susceptibility, suggesting that unlike observations *in vitro*, the parasite could induce DC activation leading to the development of Th1 cells in absence of TLR9 expression. Taken together these data show that TLR9 signaling is important for the early control of lesion development and parasite burden, but it is dispensable for the differentiation of Th1 cells secreting IFNγ, and that the high levels of this cytokine are not sufficient to control early parasite replication following *L. braziliensis* infection.

1502

DYNAMICS OF APICOMPLEXAN INNER MEMBRANE COMPLEX

Dinkorma Ouologuem, David Roos

University of Pennsylvania, Philadelphia, PA, United States

Unlike most cells, which divide by binary fission, protozoa in the phylum apicomplexa divide by a distinctive process in which multiple daughters are constructed within the mother (schizogony, endodyogeny, etc), using a membrane-cytoskeletal scaffolding known as the Inner Membrane Complex (IMC). The IMC is closely associated with the plasma membrane during interphase, but new daughters develop within the cytoplasm, establishing new IMCs. Daughter IMCs elongate rapidly, partitioning subcellular compartments according to a strict schedule. Newly assembled daughters ultimately emerge from the mother, picking up the maternal plasma membrane, and leaving behind vestiges of the maternal cell that were not incorporated into the daughters. While the maternal plasma membrane remains intact throughout this process the maternal IMC disappears -- is it degraded, or recycled to form the daughter IMC? Exploiting a fluorescently tagged integral membrane protein marker for the IMC (GAP40), we have used live cell imaging, photobleaching-recovery (FRAP), and mEos2 photoactivation to monitor the dynamics of IMC biogenesis and turnover during the replication of Toxoplasma gondii tachyzoites. We demonstrate that formation of the IMC involves two distinct steps: de novo assembly during daughter IMC elongation within the mother cell, followed by emergence from the mother cell and further maturation via recycling of the maternal IMC membrane.

1503

PLASMODIUM FALCIPARUM CDC2-RELATED PROTEIN KINASE (CRK) 4 REGULATES DNASEGREGATION AND THE ONSET OF BLOOD-STAGE SCHIZOGONY

Markus Ganter[1], Jeffrey D. Dvorin[2], Christian Flueck[3], Jing Yang[1], David A. Baker[3], Manoj Duraisingh[1]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Boston Children's Hospital, Division of Infectious Diseases, Boston, MA, United States, [3]London School of Hygiene & Tropical Medicine, London, United Kingdom

A hallmark of Plasmodium life-cycle progression is a sequence of invasive and replicative stages. Intrahepatic- and intraerythrocytic-proliferation is achieved through schizogony, where a multinucleated cell is formed after which daughter parasites bud off the mother cell. However, the regulatory proteins involved in schizogony are largely unknown. Employing the inducible destabilization domain system in a loss-of-function knockdown screen of schizont-stage kinases, we identified the P. falciparum cdc2-related protein kinase (PfCRK) 4 as essential for proliferation. Depletion of PfCRK4 leads to a complete block in early schizogony at a DNA-content of approx. 6N, which is reversible within eight hours. The block at 6N is similar to what is observed following treatment with the anti-folate drug WR99210, which might be indicative of a general cell cycle checkpoint at 6N. Despite several rounds of DNA replication, analysis by microscopy revealed that PfCRK4-knockdown parasites are unable to segregate their chromosomes. This defect is likely due to an impaired division of the spindle pole body in PfCRK4 depleted parasites. Among apicomplexan parasites, CRK4 is uniquely found in Plasmodium spp., and we provide evidence that PfCRK4 is a key regulator at the onset of schizogony.

1504

TLR7-ELICITED REGULATORY B CELLS USE IL-10 TO SUPPRESS AIRWAY INFLAMMATION THROUGH INDUCTION OF CD4+FOXP3+ T CELLS

Adnan Khan[1], Sylvie Amu[1], Sean P. Saunders[1], Casey Weaver[2], Tim Sparwasser[3], Orla Sheils[4], Padraic. G. Fallon[1]

[1]Trinity Biomedical Sciences Institute, School of Medicine, Trinity College, Dublin, Ireland, [2]Department of Pathology, University of Alabama, Birmingham, AL, United States, [3]Institute of Infection Immunology, TWINCORE, Centre for Experimental and Clinical, Hanover, Germany, [4]Department of Histopathology, Trinity College Dublin, Sir Patrick Duns Research, Dublin, Ireland

Helminths are potent modulators of the immune system using a diverse range of mechanisms. In recent times, a role for helminth-induced regulatory CD19+CD1dhi B cells (Breg) has been identified as regulators of inflammation in mouse models. Using, microarray technology we have analyzed the gene profiles of *Schistosoma mansoni*-elicited Breg. With respect to innate activation of these cells, toll-like receptor 7 (TLR7)

was a significantly upregulated pathway of interest. The use of TLR7 ligands both *in vitro* and *in vivo* demonstrated the generation of Breg - comparable to helminth-elicited Breg - that produced copious amounts of the immunosuppressive cytokine IL-10. In a mouse model of allergic lung inflammation the use of either TLR7 ligands to induce Breg or the adoptive transfer of *in vitro* generated Breg demonstrated a reduction in airway inflammation and an improvement in lung function. Previously, we have shown how helminth-induced Breg can suppress pulmonary inflammation via CD4+FoxP3+ T cells. Here, we have investigated if TLR7-elicited Breg suppresses airway inflammation via CD4+FoxP3+ T cells and whether this effect is dependent on IL-10. Our work demonstrates how deciphering mechanisms by which helminths modulate the immune system can yield specific targets of therapeutic interest.

1505

THE ROLE OF EPIDERMAL KERATINOCYTES IN THE CUTANEOUS IMMUNE RESPONSE TO *SCHISTOSOME CERCARIAE* AND THEIR EXCRETORY/SECRETORY ANTIGENS

Claire D. Bourke[1], Adrian P. Mountford[2]

Centre for Immunology and Infection, University of York, York, United Kingdom

The epidermis is the site of the initial interaction between schistosome parasites and their mammalian host. During invasion schistosome larvae (cercariae) actively penetrate cutaneous tissue via mechanical damage and the release of excretory/secretory (E/S) products containing proteolytic enzymes and glycans. Invasion promotes angiogenesis and differentiation of 'wound healing' leukocytes in the dermis but it is unclear how these events are orchestrated. Since epidermal keratinocytes are innate sensors of cutaneous wounding, we hypothesised that these cells become activated early during schistosome infection leading to changes in the cutaneous immune responses. C57BL/6 mice were exposed to live *Schistosoma mansoni* cercariae via the pinna and dermal and epidermal cells were isolated from the site of infection at 6h, 24h and 96h post-infection. Epidermal non-haematopoietic (CD45-) cells were then phenotyped *ex vivo* via flow cytometry to identify keratinocyte sub-populations. Relative to un-infected skin, a population of epidermal keratinocytes (CD45-CD326-CD34+) was found to increase following infection. The expansion of this population coincided with expression of markers associated with keratinocyte activation and wound healing in skin explants. Cultures of primary murine epidermal keratinocyte also demonstrated an activated response upon exposure to cercariae E/S material *in vitro*. The functional relevance of changes in keratinocyte sub-populations in the epidermis and their activation state was explored via analysis of parallel changes in dermis-infiltrating antigen presenting cells and tissue inflammation. These results suggest that cutaneous non-haematopoietic cells, particularly keratinocytes, may be important mediators of the early innate immune responses to schistosomiasis *in situ*.

1506

LECTIN AND C2-KINASE SIGNALING REGULATE TROGOCYTOSIS-LIKE INGESTION AND HOST CELL KILLING BY *ENTAMOEBA HISTOLYTICA*

Katherine Ralston[1], Michael D. Solga[2], Nicole M. Mackey-Lawrence[2], William A. Petri, Jr.[2]

University of Virginia, Charlottesville, VA, United States

Entamoeba histolytica is the causative agent of amoebiasis, a diarrheal disease that is a major source of morbidity and mortality in the developing world. Pathogenesis is associated with profound tissue destruction, manifesting as intestinal ulceration or extraintestinal abscesses. Parasite cytotoxic activity is central to tissue destruction, but the mechanism for killing of host cells was unknown. Recently, by employing live confocal fluorescence microscopy, we discovered that amoebae kill by biting off and ingesting distinct pieces of living human cells. The process is reminiscent of trogocytosis (Greek trogo-, nibble) between immune cells. Amoebic trogocytosis initiates within one minute of host cell contact and precedes cell death, as assessed by permeabilization and DNA fragmentation. By using imaging flow cytometry to simultaneously quantify ingestion and killing, we find that pharmacological inhibitors of trogocytosis reduce host cell death in a dose-dependent manner. Trogocytosis is relevant to disease pathogenesis, since we demonstrated using live two-photon microscopy that trogocytosis occurs during invasion of colon explants from fluorescent-membrane mice. We are currently employing dominant negative mutants and recently developed gene knockdown approaches in E. histolytica, in order to define the pathways regulating trogocytosis. Interestingly, a C2 domain-containing protein kinase, EhC2PK, is required for both trogocytosis and conventional phagocytosis in E. histolytica, suggesting that some aspects of conventional phagocytic machinery may be common to trogocytosis. We are using these and other trogocytosis mutants as valuable tools to further dissect tissue invasion and destruction in animal models of infection. Finally, it is notable that the closely related parasite E. dispar is also capable of trogocytosis, and it has been suggested that trogocytosis occurs in Naegleria fowleri. Therefore, not only do these studies change the existing paradigms for cell killing and issue destruction in amoebiasis, they also suggest an ancient origin of trogocytosis as a form of intercellular exchange.

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