## 946

### MIXED-SPECIES INFECTIONS AND ALTERNATION OF INFECTIONS OF *PLASMODIUM VIVAX* (PV) AND *P. FALCIPARUM* (PF) IN LOW TRANSMISSION AREAS: HOW DO CYTOKINES MODULATE INFECTION DYNAMICS?

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Low transmission settings provide the opportunity to study single infections, the dynamics of mixed infections and the alternation of species detected over a period of just weeks ('cryptic infections' likely mixed but only one species detected any given time). Longitudinal detection in Zungarococha, Iquitos, from 2003-2009 show that individuals with mixed infections are presenting at clinics more frequently than those with single infections (83.3% and 73.5%, respectively; p=0.15) and individuals with mixed infections report a recent febrile episode more frequently than those with PV or PF infections (73.0% and 65.4%, respectively; p=0.33). When PCR diagnosis is considered, 33.9% of mixed infections are first observed at the clinic versus 8.9% of single infections (p<0.001). This suggests that individuals with mixed infections seek treatment much earlier compared to individuals with single infections. To further elucidate the host response during these parasite interactions, the levels of ten cytokines from single, mixed and alternating infections are being determined. Preliminary data show that all microscopy-positive individuals, regardless of infection type, show an increase in IL6, IFNa, IFNg, TNF, and CRP, compared to uninfected individuals. The main difference observed between single and mixed infections is that single show a trend towards a reduction in the level of IL4 and no difference in the level of IL8, compared to controls. However, the trend in mixed infections is no difference in IL4 but a reduction in IL8. Single-species and mixed-infections tend to also have higher IL12p40 but positive infections (both PV and PF) from alternating infections show a reduction in this specific cytokine. This might be evidence that alternating species allows parasites to modulate their environment with the greatest effect that protects the parasites and the host when multiple species are co-circulating. Determining human cytokine responses are essential to understanding host-parasite interactions and the mechanisms that underlie disease severity in these regions of co-infection.

## 947

### DISTRIBUTION OF DEVELOPING GAMETOCYTE STAGES IN THE BLOOD OF MALARIA-INFECTED PATIENTS

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In the context of malaria eradication, the transmission stage (gametocyte) is of high importance, as it is the only red blood cell stage that leads to the propagation of the disease. It is well noted that mature Stage V gametocytes, but not developing gametocyte stages (I-IV), are observed in the circulating blood. It has been hypothesized that developing stages are sequestered in tissues, but we assume that prior to sequestration, the sexually committed ring stage is also present in the circulating blood. Based on published microarray data, we developed a constrained regression model to predict the distribution of three populations in a given sample: early gametocytes (rings), developing gametocytes (I-IV), and mature gametocytes (Stage V). The application of the model to published patient blood sample microarray data predicts that a subset of these samples have early and/or late gametocytes. It also predicts a depletion of developing gametocytes in all samples, as we would expect, given our hypothesis that these stages sequester. We have used this model to define a small set of stage specific markers for qRT-PCR that accurately predict the distribution of these three gametocyte subpopulations in a sample. The assay was validated using *in vitro* gametocyte development time courses, and was subsequently applied to a large number of peripheral blood samples from malaria-infected patients in Blantyre, Malawi. For a subset of these samples in which an enrichment of a gametocyte marker was observed, IFA analysis was performed for confirmation of the results. The present work sheds light on the dynamics of gametocyte disappearance and reappearance in the peripheral blood over the approximately seven to ten day sexual development. The tools presented here will facilitate prediction of patient transmission potential over the course of drug treatment, and with different combination therapies. Furthermore, the application of this methodology to tissue samples could be used to investigate the sequestration of developing gametocytes.

## 948

### MECHANISM OF BINDING OF *PLASMODIUM FALCIPARUM* TO VASCULAR ENDOTHELIUM IN CEREBRAL MALARIA

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There are an estimated 200,000 cases of cerebral malaria annually in Africa alone; mortality remains high at 20%, even with treatment. Hypoxia and tissue injury occur when large amounts of *Plasmodium falciparum*-infected erythrocytes (IE) adhere to vascular endothelium in the brain and prevent normal blood flow. Adherence of IEs is mediated by *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), a large family of clonally variant adhesion proteins encoded by *var* genes. Approximately 60 *var* gene copies are present in every parasite genome. It is unclear which PfEMP1 proteins and host receptors mediate binding to human brain microvasculature endothelial cells (HBMEC), though some studies suggest that intercellular adhesion molecule-1 (ICAM-1) is a possible host receptor. To study this binding, we took long-term parasite cultures that express a mixture of different *var* genes and a parasite line that was previously enriched for ICAM-1 binding and repeatedly panned these IE on HBMEC. We conducted initial and post-panning quantitative binding assays using common endothelial cell receptors to assess for changes in binding characteristics and applied real time-PCR to catalog *var* genes that are newly-induced in the panned lines. Initial binding assays revealed that IE that bound more highly to recombinant ICAM-1 than to other common human ligands also showed two to three times greater binding to HBMEC. Panning of non-ICAM-1 binding parasite lines on HBMEC led to upregulation of an ICAM-1 binding phenotype. We found that the genes *var6, var13, and var19* were consistently upregulated in HBMEC-panned IE. Our results suggest that a limited subset of *var* genes that encode ICAM-1 binding and potentially other phenotypes were consistently upregulated in *Plasmodium falciparum*-infected erythrocytes panned on primary human brain microvasculature endothelial cells.

## 949

### KNOWLEDGE AND COMPLIANCE OF HEALTH WORKERS TO MALARIA RAPID DIAGNOSTIC TEST GUIDELINES IN RUKUNGIRI DISTRICT, WESTERN UGANDA, 2010

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Rapid diagnostic tests (RDTs) for diagnosis of malaria were introduced in November 2008 and rolled out to all health centre (HC) of level II in Rukungiri district.. Before their introduction, health workers used

presumptive clinical means for diagnosing and treating malaria. RDTs are cost effective and reduce errors in treatment if the prescribers comply with the test results. This study assessed the knowledge and compliance of health workers to RDT guidelines for diagnosis and management of malaria in Rukungiri district. The study used both cross sectional and retrospective designs. Twenty three HCs from the entire district were selected by simple random sampling. A total of 460 cases at the selected HCs that had an RDT test for malaria were selected by simple random sampling from patient registers. Data abstracted included client particulars, RDT results and drugs prescribed. Key informant interviews were conducted with health workers. Data was analysed using SPSS version 11.0. All health workers who manage patients had adequate knowledge of RDT use. Of all 460 patients seen at health facilities, two-thirds (69%) were managed in compliance with the national RDT guidelines. 79% of the patients tested with RDTs had fever, cough, flu and headache. Patients who were more likely to receive and anti-malarial were: those that presented with fever (OR: 56.1, 95% CI: 28.5 - 110); had a positive RDT result (OR: 401, 95% CI: 142.9 - 1000); The mean compliance score for all health facilities was 10.19 (SD 1.3), median score was 10 (Range 8.0, 15). In conclusion, knowledge of health workers of the RDT guidelines was high; however, compliance was still sub-optimal. District health authorities should actively monitor the health workers' implementation of the RDT policy guidelines and respond to unmet needs.

## 950

### USING RAPID DIAGNOSTIC TESTS (RDTS) AS SOURCE OF MALARIA PARASITE DNA FOR MOLECULAR ANALYSES

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Malaria prevalence has declined drastically in most parts of Tanzania possibly due to efficient treatment regimens (using artemisinin combination therapies), use of insecticide treated bed nets and climatic changes. Although surveillance of malaria to detect the occurrence of hidden parasite reservoirs and parasite resistance to antimalarial drugs using sensitive molecular tools is important, it increasingly becoming difficult to obtain malaria positive samples from studies, such as drug efficacy trials since they cannot be performed to the same extent as before. This study was conducted to establish if sufficient DNA could be successfully extracted from rapid diagnosistic tests (RDTs) and used for various molecular analyses. Serial dilutions were made (in triplicates) from a hyper-parasitaemic sample (131,260 parasite/µl) with whole blood donated by uninfected donor and blotted on RDTs (ParaHIT®f, Span Diagnostics - India) according to manufacturers' instructions. DNA was extracted using chelex method from the three sets of RTDs (either immediately or after storage for one month at room temperature with/without silica preservatives). The extracted DNA was amplified using a nested PCR for *Plasmodium* species detection. Used RDTs (n=29) obtained from ongoing projects in Korogwe and Muheza districts were analysed to confirm initial results. Furthermore, since false negative RDTs possibly pose a problem in malaria endemic areas in transition, the detection of parasite infections among negative RDTs will be examined during a malaria survey in Muheza district planned in May 2010. DNA was successfully extracted and amplified from the three sets of RDTs. For all sets of dilutions, the minimum detection limit of malaria parasites by PCR was down to 1 parasite/µl. DNA was also extracted and successfully amplified from all 29 positive RDTs received from ongoing studies in Muheza and Korogwe districts. Results of false negative RDTs in the malariometrict survey to be conducted in May 2010 will be obtained and discussed. This study has shown that DNA can be successfully extracted from RDTs and used for detection of malaria parasites by PCR. Since the Ministry of Health and Social Welfare is planning to introduce RDTs in all health facilities, availability of used RDTs will provide an alternative source of DNA for genetic studies such as surveillance of parasite resistance to antimalarial drugs.

## 951

### QUALITY ASSURANCE OF MALARIA RAPID DIAGNOSTIC TESTS (RDT) AND ITS IMPLICATION FOR CLINICAL MANAGEMENT OF MALARIA

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In 2010 WHO changed its guidelines for the treatment of malaria to recommend parasitological confirmation in all patients suspected of malaria before treatment is started. However perceptions of reliability of malaria diagnostic tests among the frontline health care workers in resource limited settings present considerable obstacles for the effective rollout of parasitologically confirmed malaria treatment. We assessed the implications of simple measures for monitoring accuracy of malaria RDT-s in the field settings on malaria treatment practices at the outpatient clinics in Kibondo district, Tanzania. We determined the sensitivity and specificity of the malaria RDT in patients diagnosed with suspected malaria at the outpatient clinics, by comparing the results of the RDT at the clinics with microscopy done by an independent blinded laboratory technologist. Results of this investigation were shared with the clinical staff and confirmation of all patients with suspected malaria by RDT was recommended. Clinical practice was monitored by comparing the proportion of laboratory confirmed versus presumptive malaria diagnoses during the period from June to December 2007. Malaria slides and RDT's from 513 patients diagnosed in September and October 2007 in the clinics were compared. Specificity and sensitivity of RDTs used were 92.2% and 93.7% respectively. Over-diagnosis of malaria at the clinics was common and 68.8% (95% CI: 61.8% - 75.8%) of children under five years old diagnosed with malaria had a negative malaria slide. After sharing these results with the clinicians, malaria diagnoses based on laboratory confirmation in the clinics rose within two months from 38% of total malaria diagnoses to 64%. Implementing field based malaria RDT quality control measures has important implications for the clinical performance of the frontline health care providers in resource limited settings. Clinicians in rural African settings need to be convinced by disseminating and discussing evidence about RDT's from their own and similar settings in order to improve diagnostic and prescription habits.

## 952

### DEVELOPMENT OF FIELD USABLE RDT KITS FOR SIMULTANEOUS DIAGNOSIS OF MALARIA AND PREGNANCY

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Malaria is a serious parasitic disease which now occurs in more than 90 countries worldwide. In Africa, 30 million women living in malaria-endemic areas become pregnant each year. For these women, malaria is a threat both to themselves and to their babies, with up to 200,000 newborn deaths each year as a result of malaria in pregnancy. Pregnant women are more likely infected to malaria as pregnancy reduces a woman's immunity to malaria, making her more susceptible to malaria infection and increasing the risk of illness, severe anemia and death. Maternal malarial infection has serious consequences for the unborn child-it is highly to cause miscarriage, stillbirth, premature delivery and low birth weight, increasing risk of infant death. The simultaneous detection of malaria and pregnancy will help the management of complications, appropriate treatment of medication and may decrease the risk of prenatal mortality. Recently, we have developed three different types of rapid, one step qualitative assay for detecting malaria and pregnancy simultaneously. The test procedure is very simple. Just add 5 µl of whole blood to the sample well in the device and followed by adding 2 drops of assay buffer in the assay buffer well. The assay is rapid (<20 min), easy to

operate, inexpensive, portable, and have no special storage requirements and it is suitable for field situation with limited healthcare facilities and limited trained medical care staffs. *CareStart*™ Malaria /Pregnancy (pLDH/HCG) combo Test detects malaria *Plasmodium* species and pregnancy. The *CareStart*™ Malaria /Pregnancy (HRP2/HCG) combo Test detects the infection of malaria *P. falciparum* species and pregnancy. The *CareStart*™ Malaria /Pregnancy (pLDH/HRP2/HCG) combo Test distinguishes malaria *P. falciparum* species from other *Plasmodium (P. vivax, P. malaria and P. ovule)* species. These RDT kits detect less than 30 parasites/µl and as early as 1week of pregnancy.

## 953

### SHARING GOOD PRACTICES! MCTA MICROSCOPY EXTERNAL QUALITY ASSESSMENT PROGRAM FOR CLINICAL TRIALS IN AFRICA

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Microscopy remains the "gold standard" in clinical trials evaluating candidate drugs, biologics, and devices developed to prevent, treat, or diagnose malaria. Failure to perform malaria microscopy competently compromises the quality of clinical trials. False-positive diagnosis of malaria has a negative effect on the outcome of prophylactic clinical trials because the protective efficacy of the candidate product will be underestimated. Several methods of perform malaria microscopy exist but the benefits of harmonized diagnostic methods are great in multi-centre clinical trials. To standardize microscopy techniques across sites, the Malaria Clinical Trial Alliance (MCTA) supported the Kenya Medical Research Institute/U.S. Army Medical Research Unit-Kenya Malaria Diagnostics and Control Center of Excellence (MDCoE) to train microscopists and establish an external quality assessment (EQA) program in sub-Saharan Africa. The outcome was to improve competence and create sustained proficiency in preparation and staining of standardized quality slides, parasite detection and quantitation and species identification. Quarterly the sites sent 5 slides to MDCoE prepared in their laboratories with the results obtained by their microscopists. In turn MDCoE sent 5 standardized, validated slides to the sites which were read by site microscopists and results sent back. The slides provided by MDCoE consisted of negative slide, mixed species slide, and Pf parasites of differing densities. Concordance was high for parasite detection and *P. falciparum* species identification but lower for uncommon species (Pm, Po). Concordance on parasite density estimation was variable between sites and across different densities. However, all sites showed improved performance over time. In conclusion, the EQA program has demonstrated sustained proficiency by the microspcopists at trials sites and valuable lessons learned which can inform the design and implementation of future EQA programs.

## 954

### NOVEL MOLECULAR DIAGNOSTIC TARGETS FOR THE DETECTION OF *PLASMODIUM FALCIPARUM* AND *PLASMODIUM VIVAX*

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Accurate and rapid diagnosis of malaria is crucial for saving lives. Molecular diagnostic tools are important for detecting sub-clinical infections and differentiating between Plasmodium species. In spite of available genomic information for Plasmodium *falciparum* and P. vivax, the majority of PCR-based methods rely on the 18S RNA gene. While this gene has been a good target for diagnostic assays, it has certain limitations in multiplex assay platforms to detect mixed infections. Here we describe new DNA targets for the species specific detection of P. *falciparum* and P. vivax. Genomic sequences were obtained from PlasmoDB for P. *falciparum* and P. vivax and scanned with the de novo repeat finding tool RPTScout to identify potential repetitive diagnostic sequences. Candidates were screened via sequence similarity searches for vector contamination, human genome sequence, species-specificity, size and copy number, and aligned to assess conservation suitable for amplification. Repeat candidate R364 was identified in the P. *falciparum* genome and R47 was identified in the P. vivax genome. We evaluated these targets for use in PCR assays and compared them to the Snounou method, an 18S RNA gene-based nested PCR. Primers designed to candidate R364 specifically identified P. *falciparum*, and primers to candidate R47 detected only P. vivax. Both assays showed similar limits of detection to the Snounou method with a single, as opposed to nested, PCR reaction. Using known quantities of laboratory-cultured parasites, we were able to detect DNA in concentrations as low as 1parasite/µl. The method was further validated using microscopically-determined P. vivax samples from Venezuela (n=96) and P. *falciparum* samples from Tanzania (n=91). In comparison to Snounou nested PCR, preliminary data show P. vivax candidate R47 had 95.8% sensitivity and 100% specificity, and P. *falciparum* candidate R364 had 97.8% sensitivity and 100% specificity.

The novel PCR method presented here is a valuable alternative molecular diagnostic method for specifically detecting P. *falciparum* and P. vivax.

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### POINT-OF-CARE DIAGNOSIS OF MALARIA BY FLUORESCENCE LOOP MEDIATED ISOTHERMAL AMPLIFICATION

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Molecular diagnostic methods can complement existing tools to improve the diagnosis of malaria. However, they require good laboratory infrastructure thereby restricting their use to reference laboratories and research studies. Therefore, adopting molecular tools for routine clinical use in malaria endemic countries will require simpler molecular platforms. The recently developed loop-mediated isothermal amplification (LAMP)

method is relatively simple and field-amicable. In this study, we attempted to improve this method for malaria diagnosis by combining the use of a fluorescence signal (SYBR Green) as the readout and the use of a portable isothermal amplification platform with real-time fluorescence reading capability. We refer to this as the RealAmp system. Published genus-specific primers were used to test the utility of this system. Amplification was carried out at 63oC for 90 minutes with the portable reader set to collect fluorescence signals at 1 minute intervals. DNA derived from different species of malaria was used for the initial characterization. Clinical samples of *Plasmodium falciparum* were used to determine the sensitivity and specificity of the RealAmp system compared to a nested PCR method. In addition, directly boiled parasite preparations were compared with a conventional DNA isolation method. The RealAmp system was found to be simple and allowed real-time detection of DNA amplification. The time to amplification was generally less than 60 minutes. All four human malaria parasites were detected. This method detected *P. falciparum* in clinical samples with 98.9% sensitivity and 100% specificity compared to a standard nested PCR method. In addition, this method consistently detected *P. falciparum* from directly boiled blood samples (up to 40p/mL). This RealAmp system is a simple field usable tool when compared to traditional PCR methods for the molecular diagnosis of malaria and potentially other infectious agents. This tool can be used in health care settings and field laboratories and may be valuable for field use in malaria elimination programs.

## 956

### IMMUNOSENSOR DESIGN FOR DIAGNOSIS OF MALARIA USING THE RHOP-3 RHOPTRY PROTEIN OF *PLASMODIUM SP*

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*Plasmodium falciparum* causes the most lethal form of malaria resulting in approximately 500 million clinical cases annually and 1-2 million deaths in children under the age of 5 years. Currently, diagnosis of malaria depends on microscopy and is augmented by serological tests and rapid diagnostic tests. Etiological confirmation of the correct *Plasmodium* species remains challenging and molecular diagnostic tests such as the polymerase chain reaction (PCR), is not available in many malaria endemic areas. Reliable, specific and sensitive point-of care (POC) diagnostic methods that are of low cost are needed to facilitate rapid single step diagnosis of malaria. Diagnostic tests with prognostic value will increase treatment efficiency and reduce the development of drug resistant parasites in malaria endemic areas. The structure of the Rhop-3 rhoptry protein of *Plasmodium* sp. was investigated using the thermal analytical techniques of dielectric analysis (DEA), and differential scanning calorimetry (DSC) for potential immunosensor development for malaria diagnosis. The physical-chemical properties of the protein were analyzed. Antisera from patients in malaria endemic areas are highly reactive with recombinant Rhop-3 in an enzyme-linked immunosorbent assay (ELISA). The Rhop-3 protein is also secreted by the parasite and present in plasma during infection. The electrical conductivity (ps/cm) of Rhop-3 in aqueous solutions revealed bulk conductivity properties and was more reliable than surface sensor conductivity values by DEA. A change in Rhop-3 conductivity was observed in the first 5 minutes and again at 20 min in an overlay of ionic conductivity versus time, indicating a distinct electrical profile for Rhop-3. The electrical response of Rhop-3 varied by frequency at different sampling temperatures in tan delta versus frequency overlays. Calorimetry revealed unbound water crystallizing and melting at appropriate temperatures. An additional endotherm and corresponding exotherm at 14 J/g is probably related to the interaction of the protein and a "type" water. The latter may reflect specific amino acid water interactions which can be used to monitor the Rhop-3 protein in the process of immunosensor design.

## 957

### AUTOMATED REAGENT-LESS DIFFERENTIATION OF *PLASMODIUM FALCIPARUM* FROM *P. VIVAX* IN HUMAN THIN FILM BLOOD SMEARS WITH FTIR MICROSPECTROSCOPY

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In malaria cases species of infection affects course of treatment. Differentiation of *P. falciparum* from *P. vivax* by RDTs requires multiple antibodies, which increases test costs. Furthermore, RDTs are subject to reader error. Speciation by visual microscopy is dependent on the skill and availability of an expert microscopist. The objective of this study was to evaluate the utility of FTIR microspectroscopy for automatic reagent-less differentiation of *P. falciparum* from *P. vivax* infected human red blood cells. Geimsa-stained thin film blood smear slides were analyzed in this study. For *P. falciparum* positive controls, 240 slides with ring stage *P. falciparum* were prepared from culture. For *P. vivax* positive controls, 80 clinical *P. vivax* slides were collected and verified by expert microscopy (EM) and Polymerase Chain Reaction (PCR). For negative controls, 40 slides with *Salmonella*-infected blood (prepared from culture) and 40 uninfected human blood slides were prepared. Infrared spectra were measured from a small area of each slide (~12 micronsx12microns) usually containing only one red blood cell. Algorithms were written to differentiate red blood cells infected with *P. falciparum*, red blood cells infected with *P. vivax*, red blood cells infected with *Salmonella* and uninfected red blood cells based on their infrared spectra. Algorithms were tested by cross-validation. For *P. falciparum* sensitivity was 98.4 to 100% and specificity was 97.7% to 100% (95% CI). For *P. vivax* the sensitivity was 95.4% to 100% and the specificity was 98.8% to 100% (95% CI). These results suggest that FTIR spectroscopy may be useful for automated reagent-less differentiation of malaria infection. In high throughput settings spectroscopy testing may be lower cost because it does not require consumables.

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### AUTOMATED REAGENT-LESS DIFFERENTIATION OF *PLASMODIUM FALCIPARUM* FROM *P. VIVAX* IN HUMAN THIN FILM BLOOD SMEARS WITH FTIR MICROSPECTROSCOPY

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In some regions of the world malaria parasite drug resistance is present in 50% of cases. Unfortunately, tests to determine drug resistance are not clinically available forcing health ministries and doctors to make difficult choices. An economical clinical test for drug resistance would enable doctors to administer less expensive chloroquine to susceptible cases, lowering health costs and slowing the spread of resistance to newer drugs. The objective of this study was a preliminary evaluation of the utility of FTIR microspectroscopy for differentiating red blood cells infected with drug resistant strains and drug susceptible strains of *Plasmodium falciparum.* 120 Geimsa-stained thin film blood smear slides were prepared with drug-susceptible ring stage *P. falciparum* from culture (40 slides strain 3D7, 40 slides strain 1776, 40 slides D6), and 120 Geimsa-stained thin film blood smear slides were prepare with drug-resistant ring stage *P. falciparum* from culture (40 slides strain HB3, 40 slides strain Dd2, 40 slides strain 7G8). Negative controls included 40 Geimsa-stained thin film blood smear slides of uninfected human blood as well as human blood infected with *Salmonella* from culture (40 slides). Additional *P. falciparum* negative controls included 80 clinical Geimsa-stained *P. vivax* slides collected and verified by expert microscopy (EM) and Polymerase Chain Reaction (PCR). Infrared spectra were measured from a small area of each slide (~12 microns x12microns) typically containing only one red blood cell. Algorithms were written to differentiate red blood cells infected with *P. falciparum*, red blood cells infected with *P. vivax*, red blood cells infected with *Salmonella* and uninfected red blood cells based on their infrared spectrum. Algorithms were tested by cross-validation. For drug

susceptible strains, sensitivity was 97% to 100% and specificity was 98.7% to 100% (95% CI). For drug resistant strains sensitivity was 97% to 100% and specificity was 98.7% to 100% (95% CI). These results suggest that FTIR spectroscopy may be useful for automated reagentless differentiation of drug resistant and drug susceptible strains of *P. falciparum* in thin film blood smears. This capability could enable more cost effective case management and reduce the spread of drug resistance to newer drugs.

## 959

### THE EPIDEMIOLOGY OF MALARIA IN HOUSTON, 2000 - 2009

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The objective of this study was to study the epidemiology of malarial cases in Houston. All malaria reports in Houston, 1/03 and 12/09, were reviewed for demographics, site of acquisition, species, prophylaxis taken, and treatment. 115 cases from Harris County over 7 years (range 6-35/y) showed a sex ratio (M:F) of 1.96 (n=83) and mean age on presentation of 32.8. Speciation in 93 (81%) cases included 65 with *Plasmodium falciparum* (70%), 20 with *P. vivax* (22%) and 9 with *P. malariae* (10%), (1 concomitant F-M). The sex ratio for *P. falciparum* cases was 1.36. The sex ratio for *P. vivax* cases was 4.33 (n = 16, P = 0.24, Fisher's exact). The number of *P. vivax* cases decreased for the study interval (P = 0.08, test for trend). Age of onset did not differ by species. 72 of 85 with data acquired the infection in Africa. 55% (26/47 with data) of *P. falciparum* cases were reported by Nigerians. Isolates from all Central American cases speciated as *P. vivax* and 7 were from Africa. Prophylaxis was not was taken by 27/53 (51%) and was taken incompletely by 11 (21%). Prophylaxis was used exclusively by Africans (26/45, Africans vs 0/8, non-Africans, P = 0.003, 1-tailed Fisher's exact). Africans more often reported a history of travel (52/70 vs 8/17, P = 0.03, chi square) while Central American cases developed after immigration. Treatment data were available for 72 (63%), with popularly used regimens being doxycyline (23), followed by mefloquine (15) and chloroquine (12), all given most often with primaquine. In conclusion, malaria is reportedly frequently by community hospitals in Houston. Most *P. falciparum* cases are Africans who present after returning from travel to homelands. Less common *P. vivax* cases occur among non-Western sub-Saharan Africans and also Central Americans who immigrate. Attention should be directed toward completing prophylactic regimens among travelers and providing standardized therapeutic regimens (artemesinin combination therapy) currently not available in the United States.

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### POPULATION PHARMACOKINETICS AND PHARMACODYNAMICS OF CHLOROQUINE IN MALAWIAN CHILDREN WITH UNCOMPLICATED *FALCIPARUM* MALARIA

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Antimalarial pharmacokinetic variability can influence the therapeutic outcome in individuals treated for malaria. The aim of this study was to characterize the population pharmacokinetics and pharmacodynamics of chloroquine in Malawian children who received a standard dose of chloroquine alone or in combination azithromycin, atovaquone-proguanil or artesunate, for uncomplicated *falciparum* malaria. Concentration-time measurements obtained from 400 children who received the standard oral doses of chloroquine were pooled to create a dataset containing concentration data points spanning multiple dosing occasions. Pharmacokinetic parameters of chloroquine were estimated by nonlinear mixed effects modelling. We will report the mean population estimate of apparent clearance (CL/F), volume of distribution (V/F) and the variability in these parameters. An assessment of the relationship between the posterior individual estimates of pharmacokinetic parameters, *in vitro* pharmacodynamic parameters and important covariates will also be reported.

## 961

### ACCURACY OF GESTATIONAL DATING IN AN OBSERVATIONAL PREGNANCY MALARIA COHORT IN MALAWI: AN ULTRASOUND DEMONSTRATION PROJECT

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Malaria during pregnancy is associated with an increased risk for low birth weight (<2500 g). Distinguishing infants that are premature (< 37 wks) from those that are growth-restricted (<10%) requires accurate assessment of gestational age (GA). Where ultrasound (U/S) is routinely accessible, antenatal sonographic confirmation of GA is more accurate than menstrual dating alone. Our goal was to pilot the feasibility and utility of adding U/S to an observational pregnancy malaria cohort. Research staff (1 MD, 3 clinician midlevels, 1 RN) from The Blantyre Malaria Project underwent an intensive 1-week U/S training in 07/09 led by a visiting perinatologist. Didactic lectures and observed hands-on instruction focused on acquiring images of the fetal biparietal diameter, abdominal circumference, and femur length using a portable SonoSite S180 U/S machine. Following 3 months of additional practice, fetal biometric images were obtained from subjects enrolled in an ongoing malaria cohort. After electronic image review by the perinatologist, a best U/S estimate of GA was determined using standard biometric tables, and compared with menstrual dates. Of 100 pregnancies imaged, 15 women had unknown last menstrual periods; U/S therefore established GA. Of the remaining 85, U/S re-dated the pregnancy in 23 (27%) secondary to a discrepancy between menstrual and U/S dates (discrepant if > 7 days difference when imaged < 20 wks; > 14 days if 21-28 wks; > 21 days if > 28 wks). U/S demonstrated the GA to be less than anticipated in 10 and more than anticipated in 13. Images were obtainable 93.6% of the time (89.1% in the 1st 50 scans; 98% in the 2nd 50). Comparison of U/S with postnatal assessment of GA (Ballard) awaits the deliveries. In conclusion, U/S should be strongly considered in prospective malaria studies with obstetric endpoints. Reliance on menstrual dating may lead to misclassification of infants as premature (earlier due date by U/S) or as growth-restricted (later due date by U/S).

## 962

### EXPLORING THE ANTIMALARIAL EFFECT OF ANTIRETROVIRAL PROTEASE INHIBITORS IN A COHORT OF HIV-INFECTED WOMEN RESIDING IN MALARIA-ENDEMIC AREAS OF SUB-SAHARAN AFRICA

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The distribution of malaria and HIV overlap in many regions of the world. Available evidence indicates that co-infection results in increased severity of malaria and increased viral replication, potentially accelerating the course of immunosuppression, and increasing HIV transmission. Some antiretroviral protease inhibitors (PI) have been demonstrated to show a moderate antimalarial effect. To explore the clinical relevance of this we examined the incidence of malaria in a cohort of HIV-infected women as a sub-study of ACTG 5208, a study investigating the impact of previous Nevirapine exposure for PMTCT on treatment outcomes. The substudy included subjects in 6 malaria-endemic study sites across sub-saharan Africa who received alternate antiretroviral regimens, one containing the PIs lopinavir/ritonavir, the other a NNNRTI, nevirapine. Data and serum samples were collected at scheduled visits over 48 weeks. Incidence of malaria was determined by clinical diagnosis, parasitologic diagnosis by blood slide, laboratory assay for the presence of *Plasmodium falciparum* HRPII in plasma by ELISA, or LDH by Rapid Diagnostic Test (RDT), and by increase in antibody titer to the recombinant *P. falciparum* proteins AMA1 and MSP1-19. 2,971 serum samples were collected from 447 HIV infected patients. 80 subjects had a clinical or parasitologic diagnosis of malaria, among whom sera were available for 66. Interim laboratory analysis of 2,509 samples indicated a very low incidence of laboratory-confirmed malaria, with a total 7 samples positive for HRPII, in 5 individuals, 2 of whom had a concordant clinical or parasitologic diagnosis. Sera from a further 2 individuals tested positive for non-*falciparum* malaria in RDTs. Among 26 subjects with available sera and a clinical, parasitologic or HRPII antigenemia-based diagnosis, a rise in malaria-specific antibody titer was uncommon (1, 1 and 2 respectively). The lower than expected incidence of malaria in this population impeded exploration of the potential protection against malaria conferred by an antiretroviral regimen containing PIs with an antimalarial effect.

## 963

### POPULATION, BEHAVIORAL AND ENVIRONMENTAL DRIVERS OF MALARIA PARASITEMIA IN THE DEMOCRATIC REPUBLIC OF CONGO

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The prevalence of malaria in the Democratic Republic of Congo (DRC) is among the highest in the world, but there are limited data on individual and ecological risk factors for parasitemia. Real-time PCR assays were employed to detect *Plasmodium falciparum*, ovale and malariae parasites in dried blood spots collected from adult respondents to the 2007 Demographic and Health Survey, a representative sampling of the population. Using these data and the extensive questionnaire results from each of the approximately 8000 respondents, spatial statistical analyses and multilevel modeling were employed to estimate parasite prevalence and define individual and ecological drivers of infection. Of 7,778 respondents included in our models, 2,208 (28.4%) were parasitemic, with prevalence ranges from 0 to 82% between geographically-defined survey clusters across the DRC. *P. falciparum* infections were the most prevalent species, either as monoinfection (91%) or co-infecting with *P. malariae* (6%) or *P. ovale* (<1%). Using ArcGIS, parasite prevalence for all 300 GIS-linked survey clusters were input to create a comprehensive interpolated map of malaria prevalence in the DRC. Younger males were at higher risk for infection ($p < .0001$), while wealthier and more educated people who own bed nets were at lower risk ($p < .05$). Two measures of conflict were negatively associated with malaria risk ($p < .05$), suggesting that provision of antimalarial drugs by humanitarian groups in these areas may be contributing to lower prevalence. Overall, this study demonstrates the need for surveillance systems for infectious diseases that use a representative population-based sampling scheme.

## 964

### *PLASMODIUM VIVAX* MEROZOITE SURFACE PROTEIN-3 GENETIC DIVERSITY SUGGESTS ALLELE SPECIFIC IMMUNITY IN LOW-TRANSMISSION COMMUNITY COHORT

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*Plasmodium vivax* merozoite surface protein (MSP) 3 is a molecular epidemiologic marker for discerning highly polymorphic genome composite of the malaria parasite. In the present study, we sought to determine the overall *P. vivax* genetic diversity and to detect if the genotypes present in individual's first infection versus second infection of two infections occurring within 1.5 year were different. We included 65 individuals sampled in active case detection where there were two *P. vivax* microscopy infections detected from each individual, giving a total of 130 infections from Peruvian Amazon cohort collected during 2003 to 2008. *P. vivax* infection was detected by using nested PCR and then employed for restriction length polymorphism analysis using HhaI and AluI enzymes, respectively. Turnover from the first of pair-infection symptomatic case into second of pair-infection being asymptomatic was observed in 38/130 cases whereas turn over from asymptomatic to asymptomatic was observed in 28/130 cases. Each turnover cases had the highest number of prior infections before enrollment of the study, 18/38 and 20/28, respectively. Interestingly, turnover from an asymptomatic

case into symptomatic case was 14/130 and therefore showing that there is double the chance of being asymptomatic on the second infection if the person was asymptomatic at the prior infection. Across all first or second of pair-infections, MSP3 A_6_4 genotype was the most frequently observed. However in addition to previously known *P. vivax* genotypes in Peruvian Amazon community (as reported previously), there are 54 new *P. vivax* genotypes were detected. Individuals who had A_6_4 in the first and second infection were significantly less likely to be febrile in their second infection (5/6). High frequency of different genetic types in the first infection versus the second infection in the infection-pairs suggested a limitation of genetic diversity in the second infection. Moreover, individuals with a repeating A_6_4 infection were less likely symptomatic suggests allele specific immunity within this community.

## 965

### UNIVERSAL COVERAGE OF ITNS: RELATIONSHIP BETWEEN HOUSEHOLD OWNERSHIP AND USE - TANZANIA

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Insecticide-treated bednets (ITNs), a mainstay of malaria control efforts, offer both personal protection against malaria infection and community-wide protection given high levels of community use. ITN distribution schemes which aim for universal coverage (UC) may result in a higher level of use and broader community impact than targeted campaigns. However, UC has not been clearly defined and may reflect ITN ownership rather than use. We explored the relationship between ITN ownership and use through analysis of survey data from Tanzania to help inform ITN quantification for UC campaigns. The 2007-2008 Tanzania HIV/AIDS and Malaria Indicator Survey (THMIS), utilizing a two-stage sample design, is a nationally representative household survey which collected data on household ownership and use of ITNs. We defined universal household ITN "use" as the percentage of households in which all residents slept under an ITN the night prior to a survey. We created new "ownership" variables to yield proportions of households which possessed 2 ITNs per household, 3 ITNs per household, 1 ITN per 2 residents, and 1 ITN per sleeping room. We compared these household ITN "ownership" indicators with household ITN "use". A total of 8,497 households were interviewed. All residents slept under an ITN in 970 (11%) of households. Among households possessing either 2 or 3 ITNs, 34% and 32% of households contained residents in which all members slept under an ITN respectively. If ownership was defined as 1 ITN per 2 residents or 1 ITN per sleeping room, a larger percentage of all residents in a household slept under an ITN, 53% and 44% respectively. In conclusion, to achieve the transmission reduction potential of ITNs and consequent community protection, scale up of ITN distribution is needed. Among the currently used definitions for UC of ITNs, data from Tanzania suggest that 1 ITN per 2 residents best correlated with every member of a household sleeping under an ITN. These results can assist program managers in quantifying needs and distributing ITNs during UC campaigns.

## 967

### SEASONALITY AND AGE SPECIFIC MALARIA MORBIDITY IN DIDIENI, DISTRICT OF KOLOKANI, MALI

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In Mali like most of sub-saharan African countries, most of the health centers do not have capacity to confirm the diagnosis of malaria and reported malaria cases are essentially based on presumptions. This results to a lack of accurate measure of the malaria burden essential for the control and prevention strategies. To assess the place the malaria in the overall morbidity and its variation by age and season, a prospective survey was carried out in the community of Didieni from April 2007 to March 2008 including all the consultations of the resident population at the community health center in Didieni. Malaria rapid diagnostic test (Optimal®) was performed in all suspected cases of malaria all the times during the study period. Data were recorded in special records books, doubled entered and analyzed. Of the 5565 cases of consultations, 1501 (27.0%) were due malaria representing the first cause of consultations in the resident population. The frequency of malaria varied significantly with age and the season. Most of cases 91.6% (1375/1501) occurred between August and December and mainly in children under 5 years of age 43.2% (648/1501). Severe malaria represented 0.8% (47/5565) of the consultations. About 95.7% (45/47) of them occurred between August and December and mostly in children of under 5 years of age 78.7% (37/47). In conclusion, malaria continued to be the first cause of morbidity in Mali. Controls measures should target the period of August to December for impact.

## 968

### MALARIA TRENDS AMONG GOLD MINERS IN SURINAME

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Suriname has achieved the Millennium Development Goals (MDGs) in malaria in 2008 with a significant reduction of cases (>90%) and mortality (0 deaths since 2007). This was obtained scaling up integrated, locally-adapted highly effective interventions in the populations living in the interior of the country. However, gold mining has boomed in Suriname. Malaria transmission in Suriname is strongly related to population movements, being gold mining activities the main reason. This study aimed to describe the epidemiological trends of malaria among gold miners in Suriname from 2004 through 2009. The malaria surveillance system report cases among gold miners mainly in the capital Paramaribo (Tourtone clinic - TC) and malaria service deliveries working in situ in the mining areas under the coordination/supervision of the malaria program (MP). Estimated populations of 15000 - 20000 individuals have been working in gold mining areas in Suriname and additionally, 10000 in the neighboring French Guiana. Between 2006-2009, 11988 malaria diagnoses were performed and 4352 malaria cases were diagnosed. The MP diagnosed 2041 malaria cases and TC, 2311 cases. *Plasmodium falciparum* was diagnosed in more than 40% of the cases. A six fold increase in malaria cases was observed in 2009 compared with 2004. Overall 40-50%% of the malaria cases are imported from French Guiana. Eighty two percent of the individuals infected were between 20 and 50 years of age. An increased number of malaria cases in the Suriname were detected in gold mining areas between 2004 and 2009. This was due to the increased availability and accessibility of rapid diagnostic and treatment facilities in gold mining areas and an aggressive Active Case Detection policy implemented by the malaria program. Malaria prevalence in mining areas ranged from 1.1% to 4.5%. In order to control the remaining malaria foci in gold mining districts in the country, an integrated comprehensive malaria control approach will be used including long lasting insecticidal nets, IEC/BCC, aggressive active case detection and media awareness campaign. Further details of the strategy will be presented and discussed.

## 969

### HEALTH FACILITY SURVEILLANCE: AN ASSESSMENT OF DATA QUALITY AND UTILITY

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In sub-Saharan Africa, health facility (HF) data are used to monitor progress in malaria control in addition to other data sources, such as population-based surveys. Malaria case data from HFs, however, are often of uncertain quality. The U.S. President's Malaria Initiative (PMI) supports a number of HFs in 10 countries to strengthen their capacity to collect, analyze, and report on 4 core indicators: total number of outpatients, total number of suspect malaria cases, total number of suspect cases tested, and total number of lab-confirmed cases. Patient-level data are collected in 7countries and aggregate data are collected in 3 countries. PMI-supported HFs were assessed to determine the quality and utility of their data. Data were collected from 3 randomly selected HFs in each of 10 countries. Data consistency was assessed for all sites, and completeness and accuracy were assessed for those sites collecting patient-level data. Consistency was measured as the difference between HF reported summary data and the recalculated result from the assessment. Completeness was measured by counting the number of case report forms (CRF) with missing data. Accuracy was measured as agreement between the individual CRF and the HF database record. Data utility was assessed by reviewing how data were used for decision making by the HF and the National Malaria Control Program. Although 90% of the countries reported all core variables, only 30% reported data that were consistent with assessment findings. Slightly more than half (57%, 4/7) of countries captured all CRF data elements, and 71% (5/7) of countries' data were accurate. The majority of countries were able to provide examples of how data are used. In conclusion, despite the availability of dedicated PMI resources to improve HF data quality, HF data quality remains problematic, primarily due to inconsistent and incomplete data, in most of the assessed countries. Although data are being used, quality issues threaten their validity. Further efforts to improve data quality and increase data use are warranted.

## 970

### MALARIA LONG INCUBATION PERIOD, WITHOUT THE USE OF PROPHYLAXIS, IN PATIENTS DIAGNOSED OUTSIDE THE TRANSMISSION AREAS IN BRAZIL

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Brazil is a tropical country with the largest number of malaria cases in the Americas (WHO). In 2009, approximately 306,000 cases were recorded, with 99.8% concentrated in the Amazon region. In the same year, 708 cases were recorded in the extra-Amazon region, where transmission does not occur, and they are largely imported from the states of the Amazon or African countries. Prolonged incubation period in Malaria was first described by Korteweg (1902) in Holland and was subsequently observed in infections caused by some strains of *Plasmodium vivax* in temperate areas. The diversity in essential biological characteristics with the existence of two strains of *P.vivax*, or the different number of sporozoites inoculated by the bite of infectious *Anopheles* were the explanations to the differences in the duration of the incubation period and emergence of relapse in malaria. Nowadays it is mainly related to the use of prophylactic malaria treatment. The opportunity to study some cases of P. vivax malaria in Rio de Janeiro where there is no vector transmission has made it possible to study certain aspects of natural history of the disease in man, without the interference of new infections. In our study, prolonged periods of incubation (ranged from 90 to 360 days), occurred in seven (14%) patients with malaria by P.vivax as well as in one individual infected by P. malariae. The average and median of the latent period (125 days) in P.vivax infections were about nine times larger than the classical period (14 days) described in the literature. No patient had used malaria prophylaxis nor had received blood transfusions. They all came from Amazonia in Brazil, except for the patient infected by P. malariae who came from Indonesia. The disclosure of this occurrence for the first time in the tropics is particularly important because in theory, it raises changes in biological and evolutionary concepts; and in practice, because malaria is one of the most common infectious diseases among travelers and long incubation period is among the main causes of failure in malaria diagnostic suspicion.

## 971

### THE IMPACT OF MALARIA ON THE UNITED STATES MILITARY: SEPTEMBER 2001 TO PRESENT

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Malaria has had a significant impact on U.S military operations throughout history. It was responsible for greater loss of manpower than enemy fire in all conflicts occurring in tropical regions during the 20th century. Malaria continues to present a major challenge to force health protection during operations in any environment where malaria is endemic. This includes 108 countries spanning the tropical and subtropical regions of the world, including most of subSaharan Africa and large regions of South Asia, Southeast Asia, Oceania, Central Asia, the Middle East, Central and South America and the Caribbean. The U.S military is either currently deployed or has the potential to deploy on short notice to any of these regions, making malaria a leading infectious threat to mission success. In our malaria-naïve military population, an infection with any of the five *Plasmodium* species infecting humans can severely degrade performance, result in missed duty, and may lead to prolonged hospitalization and, in some cases, death. The measures used to avoid malaria frequently compromise military performance and, given the difficulties of implementing control measures and chemoprophylaxis in combat, cannot be completely relied upon to prevent infection. Recent events in a number of endemic locations including Liberia in 2003 and 2009-10, Benin in 2009, and Haiti in 2010 underscore the DoD's critical need for a malaria vaccine for deployed military personnel. This presentation will discuss the impact of malaria upon U.S. forces abroad, highlighting recent events, and will provide an introduction to the ground-breaking strides being made by military researchers in the development of a successful malaria vaccine to protect deployed forces.

## 972

### A COST ANALYSIS: A MALARIA OUTBREAK AMONG MILITARY PERSONNEL DEPLOYED TO LIBERIA

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"The history of malaria in war might almost be taken to be the history of war itself" wrote Col. C. H. Melville of the Royal Army Medical College, London in 1910. A century later, malaria continues to be a major threat to every military operation which occurs in malaria-endemic areas. The lost work hours, impact on force readiness, risk to mission accomplishment, financial toll, suffering and occasional tragic deaths of young able troops despite the availability of effective insecticides, repellants and

chemoprophylactic drugs are constant reminders that the problem of malaria in the military remains to be solved. This ongoing threat to deployed personnel was highlighted in 2003 when 28% of a 290 person Joint Task Force was stricken with *Plasmodium falciparum* malaria infection despite being prescribed mefloquine for prophylaxis, necessitating the airlift evacuation of 44 U.S Marines to Germany or the U.S., five of whom required admission to an intensive care unit (ICU). A total of 41 Marines were evacuated to the National Naval Medical Center (NNMC) in Bethesda, MD including three of the five individuals requiring ICU support. A retrospective record review was performed to assess the costs associated with the evacuation and hospitalization of the 41 Marines. The results of this review will be presented as a 21st century benchmark of the magnitude of costs associated with malaria outbreaks in deployed troops. It is our intent that this estimate of the financial impact of a malaria outbreak will serve to assist with the calculation of a return on investment (ROI) for resources invested in new products to protect military personnel against malaria, support military planning for future deployments in endemic areas and inform funding decisions based on disease impact.

## 973

### PREVALENCE OF *PLASMODIUM VIVAX AND P. FALCIPARUM* IN PREGNANT WOMEN OF THE PERUVIAN AMAZON, A LOW TRANSMISSION ZONE

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The Amazonian communities surrounding Iquitos, Peru are hypoendemic for *Plasmodium vivax* and *P. falciparum*. In recent years, the prevalence of infection of both parasites has been decreasing in the general population; however, the prevalence in pregnant women is unknown. Malaria in pregnancy (MIP) poses serious risks to a pregnant woman and her fetus, increasing risks of maternal anemia and infant low birth weight. The objectives of this study were to determine the regional burden of MIP, assess whether or not pregnant women are at increased risk for either or both species of malaria, and ascertain whether the trend in pregnant women is congruent with the decreasing incidence of malaria in the general population of the Peruvian Amazon basin. From 2004-2008, 950 women were enrolled in the study at the time of delivery. *P. falciparum* and *P. vivax* parasites were detected using both standard microscopy techniques and polymerase chain reaction (PCR). Enzyme-linked immunosorbent assays (ELISAs) were performed against recombinant merozoite surface protein1-19 (MSP1-19), an erthyrocytic stage protein for both *P. falciparum* and *P. vivax* to determine the existences of IgG and IgM in patient serum. Most women (*P. vivax* 58.7%, N=917; *P. falciparum* 22.2% N=898) had a previous lifetime report of malaria (clinical, symptomatic). Preliminary data indicates that the prevalence of *P. vivax* (pregnant 15.2%, N=204; non-pregnant 9.2%, N=2098) and *P. falciparum* (pregnant 7.8%, N=204; non-pregnant 4.3%, N=2098) were higher in our pregnant than non-pregnant cohort from 2003-2008. Unlike previous studies (in high transmission zones), our low transmission study found that primigravids were not more likely than multigravids to have *P. vivax* (ages 15-19 p=0.4366; ages 20-30 p=0.9562) or *P. falciparum* (ages 15-19 p=0.1421 chisquared; ages 20-35 p=0.8232 chisquared) when controlled for age. Analysis on ELISA data is pending. The current analysis indicates that MIP in low transmission zones may behave differently than in high transmission zones. This has implications for health policy and control methods in hypoendemic areas.

## 974

### MALARIA EPIDEMIOLOGY IN A SUB-URBAN AREA OF THE PERUVIAN AMAZON REGION

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Vivax malaria is endemic in the Peruvian Amazon region where it represents up to 80% of all malaria. Intensive and regular malaria control activities have been carried out since the end of 2006 leading to a decrease in the overall malaria prevalence. However, malaria remains a public health problem, and in addition, recent publications from the Amazon region have reported high numbers of asymptomatic and sub-patents infections. The objective of this study was to estimate the prevalence of patent and sub-patent malaria infections and their epidemiological characteristics in a peri-urban community of Iquitos city the capital of the Peruvian Amazon Region. A cross-sectional survey was conducted in Ex Relleno, a newly settled community with low socioeconomic status. Villagers were invited to be examined and treated for malaria infections by a medical doctor, on a voluntary and free of charge basis. After clinical examination, a finger prick blood sample was taken for microscopic and molecular examination (species-specific PCR). A total of 169 individuals were examined, among which only 4 (2.4%) were found positive for *Plasmodium spp* by microscopy (patent infections), while another 20 infections were identified when using PCR, leading to a malaria prevalence of 24 (14.2%). *P.vivax* prevalence was 2.4% (4/169) and 3.6% (6/169), respectively by microscopy and PCR technique, whereas for *P.falciparum* these figures were respectively, 0% (0/169) and 10.7% (18/169). Patent infections were mainly symptomatic (3/4) while all *P.falciparum* PCR positive individuals were asymptomatic. In conclusion, most of the malaria infections in this sub-urban community were sub-patent and asymptomatic. Even though sub-patent infections might be less infectious to malaria vectors as compared to patent infections, this reservoir, by its size and its hidden nature, is likely to be a play a major role in malaria transmission and will complicate further elimination strategies in the Amazon region. Further research is necessary to assess the extent of this phenomenon and the long term impact of different interventions.

## 975

### LOW PARASITE MULTIPLICATION RATE WAS OBSERVED IN LESS COMPLEXITY OF INFECTION COMMUNITY PERUVIAN AMAZON

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The most dangerous type of malaria specie is *Plasmodium falciparum*, however this infection is fast in the human is hard try to culture *in vitro*, and with this obstacle difficult to do assay and gain more knowledge about the pathways for the invasion to red blood cells and what is the selectivity that has the parasite to enter the RBC and what are the alternatives ways to infect the cells. For this study we included 50 samples from persons with malaria *Falciparum* from Iquitos Nauta road, Loreto. Communities with low transmission for malaria but constant all the year, these samples were collected since 2005-2008. All these samples were entered to the culture to see the SI in the day 0 and day 2 and the impact in the success and the relation with the clinical manifestations. The samples were entered to the culture was from people who had 26 in average years old, strains showed geometric mean SI at day cero 2.30 and day two geometric mean 3.69, also the age average for the SI > 1 29 years old, PMR mean 0.06. There was no difference between SI>1 (2/14) and SI<1 (4/36) in terms of patients presenting more than 5

symptoms. However there was significant difference observed between patients presenting less than symptoms (2/14) as compared to 5 and more symptoms (12/14) when SI was less than one.

## 976

### OVERWHELMING MALARIA PREVALENCE IN CAMEROONIAN SCHOOLCHILDREN

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Malaria remains the main children killer in sub-Saharan countries in 2010 despite numerous efforts to tackle the disease. Control programs including distribution of ITNs to pregnant women and delivery of IPT has widely been adopted in endemic regions. Fetching parasitological data is crucial for any control strategy and this has become easier with the use of battery-operated fluorescence microscopes on the field. Nevertheless, school children who constitute one of the main targets are not yet focused enough for malaria control interventions. The objective of this study was to conduct a cross-sectional survey to assess malaria prevalence in schools of semi-urban and rural areas in south-west Cameroon in order to propose integrated control measures. 542 primary school children in rural and relatively urbanized areas aged 6 to 14 years were screened in 4 schools during 4 days in April 2010. The inclusion criterion was the handing over of an informed consent form signed by parent/legal guardian. Demographic and clinical data were recorded. Blood was collected by finger prick. Parasitaemia was assessed on the spot using Partec Rapid Malaria Tests slides and 3 CyScope® fluorescence LED microscopes operated with built-in rechargeable batteries (PARTEC, Görlitz, Germany). There were 1367 total school children with 542 participants (participation rate = 39.65%). The number of positive cases among the participants was 313 or a rate of 56.7%. Prostration, fever, headache, abdominal pains were the most common symptoms for children with high parasitaemia (7.7 % of tested children). Malaria prevalence remains extremely high in semi-urban and rural schools of the Buea Health District. The low adhesion rate may be due to superstitious believes of many parents who link children's blood collection to witchcraft. The results can be biased because consenting parents may have more frequent malaria cases in their family. In conclusion, sensitization should be intensely conducted and rapid malaria screening encouraged in schools for a better control of malaria.

## 977

### *PLASMODIUM FALCIPARUM* MULTIPLICATION RATE AND SURVIVAL OF PARASITES IN *IN VITRO* CULTURE IS ASSOCIATED WITH *IN VIVO* INFECTION CLINICAL FACTORS, BLOOD GROUP, AND PARASITE GENETIC DIVERSITY

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To better understand the factors influencing *Plasmodium falciparum* success in *in vitro* culture, a study was designed to investigate epidemiologic variables on culture success over time. Host variables considered upon collection of infected blood were: age, sex, temperature, hematocrit, and blood group. Parasite variables considered, included: parasite density, complexity of infection (COI), and genotype. In this study, 306 isolates were collected from 2003-2009, within Amazonian villages near Iquitos, Peru, though passive and active case detection. Parasites from vacutainer blood were cultured *in vitro*. Parasite growth over time was divided into 5 categories based on the %Δ in parasitemia. Host and parasite variables were analyzed independently and stratified by age and COI. Growth rate in first 48hrs (multiplication rate: "MR") and culture success over time was studied in relation to host and parasite variables. *P. falciparum* cultures with the greatest success were cultured from individuals with a higher parasite density ($p<0.0001$). Complex infections successfully adapted to culture at a higher frequency than single infections ($p<0.032$) and COI was also correlated to increased disease severity in children ($p<0.001$). Genotype analysis indicated that the Mad20 allele-type of *Pf*msp1-block 2, correlated to increased disease severity, which then translated into increased culture success. Patient isolates with blood groups other than O (A, B, AB) had a greater MR in the first 48hrs of culture ($p<0.016$). In conclusion, results from this study indicate variables that might reflect host immunity or pathology of parasitemia *in vivo* are related to the MR and growth *in vitro*. Individuals of the O blood group might induce a strong selective pressure that limits parasite growth. Finding a relationship between genetic diversity and MR might suggest associated parasite factors that impact growth or an ability to invade host RBCs. This study will be further investigated using other genetic markers and more detailed analyses of clinical symptoms.

## 978

### BIOPHYSICAL AND IMMUNOLOGICAL STUDIES WITH A RECOMBINANT *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE PROTEIN (PFCSP)

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A highly purified preparation of a near full length recombinant circumsporozoite protein (PfCSP) was analyzed by SDS-PAGE and shown to migrate ~10 kDa higher than its predicted 30 kDa molecular weight under both reducing and non-reducing conditions. Blue-native non-denaturing PAGE and analytical size exclusion chromatography further confirmed that the rCSP monomer had a highly extended molecular structure and its size was equivalent to a ~60 kDa globular protein. Vaccination of PfCSP along with an adjuvant Montanide ISA720 induced high titer antibodies in mice and this vaccination conferred sterile protection in two strains of mice against challenge with a transgenic *Plasmodium berghei* parasite line that expressed the *P. falciparum* CSP gene. The anti-PfCSP antibodies recognized the native CSP on sporozoites by IFA and inhibited the invasion of NF54 strain sporozoites into hepatocytes. Availability of a high quality near full-length rCSP and the transgenic mouse protection model will allow us to develop novel strategies to enhance the protective efficacy of CSP based vaccines in humans.

## 979

### A PHASE 1B DOUBLE-BLIND RANDOMIZED CONTROLLED AGE-DEESCALATING TRIAL OF TWO VIROSOME FORMULATED ANTI-MALARIA VACCINE COMPONENTS ADMINISTERED IN COMBINATION TO HEALTHY SEMI-IMMUNE TANZANIAN ADULTS AND CHILDREN

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Influenza virosomes represent an innovative antigen delivery system that has already proven its suitability for vaccine design. The aim of this trial was to demonstrate the safety and immunogenicity of the combination of two virosome formulated malaria peptidomimetics (PEV3B: 50 µg AMA-1

and 10 µg CSP) in semi-immune subjects. The design was a prospective randomized, double-blind, controlled, age-deescalating study. PEV3B was injected i.m. on days 0 and 90. Control vaccine was Inflexal V (virosomal influenza vaccine). Specimens for humoral response were obtained at screening, and on days 30, 90, 120, 180, 365. 10 adult males and 40 children aged 5-9 years living in a malaria endemic area were recruited, 8 adults and 32 children were injected with PEV3B, 2 and 8 respectively with Inflexal V. No serious or severe adverse events related to the vaccine were observed. The only local solicited adverse event reported was pain at the injection site. The incidence of pain was higher in the Inflexal V group compared with the PEV3B group (50% vs 10%, p=0.01). General solicited adverse events reported were headache and elevated temperature, with comparable rates between groups. For immunogenicity, antibody titers after vaccination were always higher in the PEV3B group than in the Inflexal V one at all sample days for both antigens ($p<0.05$), except at day 120 for CSP in adults, and day 365 for AMA-1 in children. In children the proportion of responders using either antigen was significantly higher in PEV3B than in Inflexal V ($p<0.05$), except for AMA-1 at Day 365. Incidence rate of clinical malaria from day 120 (30 days post second vaccination) until day 365 was half in children injected with PEV3B than with Inflexal V (0.00342 vs 0.00178, p=0.09). The safety data demonstrated that 2 vaccinations with PEV3B are safe and well tolerated. PEV3B elicited long-lived humoral responses to both target antigens with the strongest response generally observed 30 days after second immunization. This study confirms that virosomes are a suitable delivery system for malaria peptide antigens in malaria semi-immune subjects, including children.

## 980

### PROGRESS ON THE DEVELOPMENT OF A SECOND GENERATION PAN-REACTIVE APICAL MEMBRANE ANTIGEN-1 VACCINE

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Apical Membrane Antigen-1 (AMA1) based vaccines have shown promising parasite inhibitory effects against malaria in Phase 2a/b clinical trials. The major hurdle in the path of AMA1 vaccine development remains the diversity of its field isolates and the strain-specificity of its immune response. The availability of the crystal structure of AMA1 has allowed us to follow a two-pronged strategy to design a second generation pan-reactive AMA1 vaccine. First, we are using growth inhibition assay with polyclonal and monoclonal antibodies to map the cross-reactive inhibitory epitopes of AMA1. We hypothesize that cross-reactive epitopes displayed on an immunologically silent scaffold can form the basis of an engineered vaccine that will induce broadly inhibitory antibodies against AMA1. Alternatively we are also using the available structural and phylogenetic data on AMA1 to choose alleles that can be included in a rationally designed polyvalent vaccine. Results of cross-reactive epitope mapping, rabbit immunogenicity and parasite sero-typing using growth inhibition assays will be presented. Although AMA1 is being used here as a model to test strategies to broaden immune responses, similar approaches may be applicable to other infectious diseases where diversity remains a major hurdle to vaccine development.

## 981

### REVIEW OF THE HUMAN MALARIA CHALLENGE MODEL AT THE WALTER REED ARMY INSTITUTE OF RESEARCH FROM 1995-2007

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The seminal paper first describing the success of the human malaria challenge method was published in 1986 by Chulay et al. In this model, female *Anopheles* mosquitoes fed on *in vitro* cultures of *Plasmodium falciparum* driven to gametocyte production, and then fed on six malaria-naïve volunteers. The Walter Reed Army of Research has been conducting the human malaria challenge since the Chulay study, and the Department of Entomology has provided such mosquitoes for challenges involving over 1000 volunteers. The safety and clinical outcomes of the malaria challenge model in clinical trials have been reviewed in two publications. Church et al reviewed records of 18 malaria challenge studies between 1985 and 1992, and Epstein et al discussed studies conducted from 1996-2002. This study adds to the data available collecting data from 12 studies conducted at Walter Reed Army Institute of Research from 1995 to 2007 not previously reviewed. This is thr largest review to date and involves approximately 550 human volunteers who were experimentally challenged by the bite of *Anopheles stephensi* mosquitoes infected with *P. falciparum* sporozoites including volunteers after receiving one or more malaria vaccinations or control volunteers. Our goals will be to summarize the safety data of the malaria challenge model by delineating the signs and symptoms reported. We will report the frequency, severity and duration of these symptoms as well as describe the laboratory results and identify any abnormalities wherein associated with malaria challenge. In addition we will define parasitologic infections in challenge volunteers by prepatent, patent and incubation period, any relationship of the clinical disease and time of parasitemia, and further quantify the details of malaria infection in vaccine-protected, non-protected, and non-vaccinated volunteers. With the increasing cost and complexity of overseas trials to assess vaccine efficacy, the challenge model is of increasing importance in evaluating which malaria vaccines should go on to field trials.

## 982

### FORMULATION AND PRE-CLINICAL EVALUATION OF TRANSMISSION BLOCKING POTENTIAL OF PLANT-PRODUCED *PLASMODIUM FALCIPARUM* SEXUAL STAGE PFS25 AND PFS230 VACCINE CANDIDATES

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Malaria is a serious and sometimes fatal mosquito-borne disease caused by a protozoan parasite. There are hundreds of millions of cases of malaria occurring each year around the world, and nearly one million people are killed. Malaria is transmitted by the female *Anopheles* mosquito which takes up the sexual stage of the parasite during a blood meal. The parasite completes the sexual stages in the mosquito before being transmitted to a

subsequent host. Vaccines directed against the mosquito parasitic stages are designed to halt development into oocysts and thus are transmission-blocking vaccines. We are targeting different antigens for development of an effective transmission blocking vaccine and have successfully produced multiple versions of the Pfs25 and Pfs230 antigens in our plant-based launch-vector system and have shown them to generate strong transmission blocking activity. We are currently evaluating multiple versions of our antigens in dose ranging and adjuvant studies. Results of these studies will determine a candidate vaccine for clinical development.

## 983

### PROTECTIVE IMMUNE RESPONSES ELICITED BY IMMUNIZATION WITH A CHIMERIC BLOOD-STAGE MALARIA VACCINE PERSIST BUT ARE NOT BOOSTED BY *PLASMODIUM YOELII* CHALLENGE INFECTION

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*Plasmodium falciparum* merozoite surface protein 1 has emerged as the lead blood-stage vaccine candidate with *Pf*MSP142 the most advanced. Preclinical studies showed that protection afforded by r*Pf*MSP142 depended on the induction of high levels of neutralizing antibodies against epitopes in the C-terminal EGF-like domains of MSP119. Disappointingly, *Pf*MSP142 vaccine has failed to confer acceptable protection in humans, partly due to, T and B cell epitope polymorphisms and overall poor immunogenicity. Moreover, the likelihood that a subunit vaccine will successfully control this complex parasite is being questioned. We have investigated some of these issues using the murine malaria parasite, *Plasmodium yoelii*. We focused on improving the design of the vaccine construct by coupling the protective *Py*MSP119 with the conserved and relatively immunogenic epitopes of *Py*MSP8 to generate chimeric *Py*MSP1/8. We previously reported that immunization with r*Py*MSP1/8, formulated in Quil A adjuvant, protected against lethal *P. yoelii* 17XL, well beyond that achieved by single or combined immunizations with the component antigens. Here, we continue the evaluation of the chimeric *Py*MSP1/8 vaccine and show that immunization with r*Py*MSP1/8 elicited an MSP8-restricted T cell response that was sufficient to provide help for both *Py*MSP119 and *Py*MSP8 specific B cells to produce high and sustained levels of protective antibodies. The enhanced efficacy of immunization with r*Py*MSP1/8, in comparison to combined formulation of r*Py*MSP142 and r*Py*MSP8, was not due to an improved conformation of protective B cell epitopes or creation of novel protective epitopes. Unexpectedly, r*Py*MSP1/8 vaccine-induced antibody responses were not boosted by exposure to *P. yoelii* 17XL infected RBCs. However, r*Py*MSP1/8 immunized and infected mice mounted robust responses to a diverse set of blood-stage antigens. The data support the further development of *P. falciparum* MSP1/8 chimeric vaccine but also suggest that vaccines that prime for responses to a diverse set of parasite proteins will be required to maximize vaccine efficacy.

## 984

### PHASE 1/2A CLINICAL TRIAL ON SAFETY, TOLERABILITY, IMMUNOGENICITY AND EFFICACY OF PRIME BOOST REGIMEN OF DNA- AND ADENOVIRUS-VECTORED MALARIA VACCINES ENCODING *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE PROTEIN (CSP) AND APICAL MEMBRANE ANTIGEN (AMA1) IN MALARIA-NAÏVE ADULTS

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Malaria causes approximately 515 million cases and 1 million deaths annually. Genetically-based vaccines such as DNA plasmids and adenovirus vectors induce strong CD8+ T cell-mediated immunity, believed to be important in protection against the hepatic stage malaria. Heterologous prime-boost regimens may overcome the effects of pre-existing immunity to viral vectors. This trial assessed the safety, immunogenicity and efficacy of a prime-boost malaria vaccine in healthy, malaria-naïve adults. Three doses of a DNA vaccine consisting of two plasmids (Vical Inc.), encoding CSP and AMA1 (1 mg each), were delivered intramuscularly by jet injection (Biojector 2000 Inc.) at four-week intervals. Sixteen weeks later, a boosting dose of an adenovirus-vectored vaccine (AdCA) was given intramuscularly by needle. The AdCA consisted of two serotype-5 adenovectors (GenVec Inc.) encoding CSP and AMA1 (1 x e10 pu each). Four weeks following the AdCA boost, 15 immunized subjects and six unimmunized controls were challenged with homologous *Plasmodium falciparum* sporozoites via five infected-mosquito bites. Both the DNA and AdCA vaccines were found to be safe and well-tolerated. There were no vaccine-related serious adverse reactions. All controls and eleven immunized subjects developed parasitemia. There was no significant delay in parasitemia between groups. Four immunized subjects (26.7%) remained asymptomatic and sterilely protected 28 days post-challenge. Three protected subjects had a strong cell-mediated immune response to AMA1 or CSP + AMA1 as determined by ELISpot. Preliminary results from CD4/CD8 depletion study and flow cytometry study will be presented. In conclusion, the results from this trial demonstrate proof of principle regarding the efficacy of a DNA-prime, Adenovirus 5-boost vaccine regimen against *falciparum* malaria. Further study is needed to compare efficacy of the full vaccine regimen to that of the individual vaccine components.

## 985

### INTERFERON GAMMA ELISPOT RESPONSES IN PERIPHERAL BLOOD MONONUCLEAR CELLS FROM VOLUNTEERS IMMUNIZED WITH A METABOLICALLY ACTIVE, NON-REPLICATING PLASMODIUM *FALCIPARUM* SPOROZOITE VACCINE

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Protection of mice by immunization with radiation-attenuated sporozoites is dependent on T cells and interferon gamma (IFNγ). The aim of this work was to determine if immunization of volunteers with the PfSPZ Vaccine, a metabolically active, non-replicating *Plasmodium falciparum* (Pf) sporozoite (SPZ) vaccine, might likewise induce PfSPZ-specific IFNγ responses, and if these responses might correlate with protection. We developed an ELISpot assay that utilizes live PfSPZ (produced and cryopreserved in an identical fashion to the vaccine itself), instead of individual proteins or peptides, to stimulate peripheral blood mononuclear cells (PBMCs) in culture. The rationale behind using whole sporozoites as antigen in the ELISpot as opposed to individual peptides or proteins was that hundreds to thousands of proteins may be expressed by sporozoites, and it is not known which ones are responsible for protective immunity after immunization with radiation attenuated sporozoites and thus which could be selected for use in a more traditional ELISpot assay. Fresh PBMCs collected prior to immunization and 2 weeks after the fourth, fifth, and sixth doses of PfSPZ Vaccine were incubated with irradiated (150 Gy) PfSPZ for 36 hours in culture, and the IFNγ spot forming cells were enumerated. Post-immunization PBMCs from more than 50% of volunteers satisfied the criteria set forth for a positive response, demonstrating that immunization of the volunteers with the PfSPZ Vaccine induced PfSPZ-specific T cell responses. The magnitude of the PfSPZ-specific IFNγELISpot responses increased with increasing doses of the PfSPZ Vaccine and there was no significant difference between responses in volunteers immunized by the intradermal or subcutaneous routes. Surprisingly, the highest responses were noted after the fourth dose of the vaccine and did not increase after the fifth and sixth doses. The use for the first time of sterile, irradiated, purified PfSPZ as antigen in an IFNγ ELISpot assay has generated clear data regarding T cell responses in humans immunized with the PfSPZ Vaccine.

## 986

### THE EFFECTS OF ROUTE OF DELIVERY ON PROTECTIVE EFFICACY OF RADIATION ATTENUATED *PLASMODIUM YOELII* SPOROZOITES IN THE MURINE MODEL FOR THE PFSPZ VACCINE

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Immunization of humans with attenuated *Plasmodium falciparum* sporozoites is the gold standard for the induction of sterile immunity against malaria and is the scientific rationale for commercial development of the PfSPZ Vaccine, currently in phase I clinical trials. While the routes of vaccine administration and dose size for the current trial had to be empirically chosen for safety and pragmatic reasons, the optimal dose, dosing regimen and route of delivery for high-level protection are still unknown. We have conducted studies using the murine model for malaria in order to identify a method that provides a high degree of protection with the lowest number of sporozoites. Mice were immunized with radiation attenuated *P. yoelii* sporozoites (irrPySPZ) by various routes and the most efficacious route of delivery of sporozoites was found to be intravenous; 3 doses of just 750 irrPySPZ each usually conferred 90%-100% protection. When irrPySPZ were administered subcutaneously (SC) or intradermally (ID), a 6-7 fold increase in dose was required for comparable protection. When irrPySPZ that had been cryopreserved using a method similar to that for the PfSPZ Vaccine were used for immunizations, a 2-3 fold increase in irrPySPZ administered by the intravenous route was required for comparable protection to that of fresh irrPySPZ administered by the same route, and a 3-5 fold increase in numbers of irrPySPZ administered by ID or SC routes as compared to fresh irrPySPZ. Thus, compared to non-cryopreserved irrPySPZ administered intravenously, a >20-fold increase of cryopreserved irrPySPZ administered ID or SC was required for a high degree of protection. These results suggest that the protective potential of the PfSPZ Vaccine can be best demonstrated with intravenous inoculation, while at the same time parenteral administration for mass immunizations can be targeted to closely mimic the intravenous route using variations in injection techniques.

## 987

### DEVELOPMENT OF A *PLASMODIUM VIVAX* RECOMBINANT CS PROTEIN VACCINE

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*Plasmodium vivax* (Pv) causes 80-250 million cases of malaria annually, as many cases of malaria in travelers as *P. falciparum* (Pf), severe morbidity and mortality, and substantial economic burden. There is a huge potential market for a Pv vaccine in travelers and military from the developed world, and among populations in countries with endemic Pv. The fact that Pf and Pv co-exist in most malaria endemic areas presents technical and ethical constraints for deployment of a vaccine effective only against Pf, and malaria cannot be eradicated without eliminating Pv. The only subunit malaria vaccines that have been reproducibly shown to prevent Pf malaria in humans are based on the Pf circumsporozoite protein (PfCSP). These vaccines elicit antibodies against the central repeat region of the molecule, which is conserved in all isolates of Pf. Development of a PvCSP vaccine has been complicated by the fact that there are 2 major alleles 210 and 247, based on variation in sequence of the central repeats. We constructed PvCSP recombinant proteins that combined 3 copies of PvCSP 210 repeats and 3 copies of PvCSP 247 repeats with N-terminus, C-terminus or N- and C-termini (full length) of the PvCSP. All were expressed in Pichia pastoris as secreted proteins. All induced antibodies in mice that recognized PvCSP as well as native protein on air-dried Pv sporozoites expressing PvCSP 210 (India) or 247 (Thai) in IFAs. All were recognized by sera from individuals from Pv-endemic areas. The induced antibodies were biologically active as they inhibited invasion and development of Pv sporozoites in hepatoma cells. At a 1:20 dilution, these antibodies had a range of 78 - 84% inhibition as compared to 95% inhibition with 100 µg/mL of the protective mAb, NVS3. Further, mice immunized with the recombinants had good T cell responses against the PvCSP as measured by IFNγ ELIspot assays. Comparative assessments selected the full length PvCSP as the vaccine candidate. Progress on process development, manufacturing and characterization in compliance with cGMPs will also be presented.

## 988

### DEVELOPMENT OF *PLASMODIUM FALCIPARUM* CELTOS AS A MALARIA VACCINE IMMUNOGEN

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The conserved *Plasmodium* protein CelTOS (cell traversal protein for ookinetes and sporozoites) mediates the invasion of sporozoites through the liver sinusoidal cell layer and the migration of ookinetes through mosquito mid-gut epithelial cells. Thus, it has the potential to induce protective immune responses against pre-erythocytic and mosquito stages of the malaria parasite, thereby preventing infection and transmission. To characterize the biological activity of immune responses against CelTOS and to move toward development of a *P. falciparum* (Pf) CelTOS immunogen we expressed, purified and characterized Pf and P. yoelii (Py) CelTOS in Pichia Pastoris. We next studied in Balb/c and/or CD1 mice the immunogenicity of the recombinant proteins alone with the following adjuvants, GLA-SE emulsions (with TLR4 and 9 agonists), Montanide 720, TiterMax and Freunds, and as part of a prime boost strategy using attenuated adenovirus serotype 5 (Ad). IgG responses to the antigen in mice immunized with recombinant protein alone revealed OD 1 titers of greater than > 100,000 by ELISA and end point titers of 12,800 in IFAs of air-dried Pf and Py sporozoites showing that antibodies recognized native CelTOS. Antibodies against PfCelTOS were biologically active and inhibited the development of liver stage parasites in ILSDA assays (49% reduction in liver invasion compared to adjuvant control sera). The antibodies against PfCelTOS recognized the protein in Pf retorts and ookinetes by IFA, and are now being studied for transmission blocking activity. Further, Interferon Gamma (IFNγ) ELIspot data showed increased T-cell responses from spleen cells isolated from mice immunized with PfCelTOS in TLR9 emulsion (207 Net SFCs/106 against 14-mer peptides of PfCelTOS, 93 net SCFs/106 against recombinant PfCelTOS) compared to those immunized with emulsion alone. The protective efficacy of recombinant PyCelTOS in mice is being assessed alone and as part of the prime-boost strategy with adenovirus expressing PyCelTOS. The recombinant PfCelTOS is now in process development to be used as an immunogen alone or in combination with other pre-erythrocytic immunogens like PfCSP in clinical trials.

## 989

### BIOASSAY TESTING WITH PERMANET-2 LONG LASTING INSECTICIDAL NET SAMPLES COLLECTED AFTER 3 TO 32 MONTHS OF USE IN ETHIOPIA DEMONSTRATES PERSISTENCE OF INSECTICIDE ON NETS BUT REDUCED KILLING EFFECT IN WILD TYPE *ANOPHELES ARABIENSIS*

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After large-scale distribution of PermaNet2 long-lasting insecticidal nets (LLIN) in Ethiopia in early 2007, nets were collected from households to assess insecticide concentration and effectiveness at killing *Anopheles* mosquitoes at 3-6 months (150 nets, 3 sites), 17-21 months (200 nets, 10 sites) and 28-32 months (220 nets, 11 sites) after distribution. Nets for bioassays were randomly selected from these samples. Deltamethrin concentration was assessed by X-ray fluorescence spectroscopy. Bioassays used the CDC modified WHO cone method, with a 3 minute exposure of 30-52 mosquitoes per sample in 8 replicates, followed by a 24 hour holding period. For the 3-6 month samples, bioassays were done in Atlanta using the deltamethrin susceptible *An. gambiae* Kisumu strain. Subsequent bioassays were done at Adama, Ethiopia against the susceptible *An. arabiensis* Nazareth strain and adults reared from wild caught *An. arabiensis* larvae collected at Sodere, Oromia Region. Results were adjusted for control tests (untreated netting) run simultaneously. In tests with *An. gambiae* after 3-6 months of use, the average adjusted 24 hour mortality was 99.4% (N=24 nets, range 92.3-100%). After 17-21 months of use (N=40 nets), the average mortality was 92.7% (range 33.3-100%) with the susceptible Nazareth *An. arabiensis* and 90.4% (range 61.9-100%) with wild caught mosquitoes. After 28-32 months (N=44 nets), the average mortality was 94.6% (range 68.4-100%) with the susceptible strain but had declined to 46.1% (range 0-90%) with wild caught mosquitoes. The mean (range) deltamethrin concentration on the tested nets was 61.5 mg/m2 (range 8.6-97.3) after 3-6 months, 43.3 (1.4-93.7) after 17-21 months, and 44.3 (13.1-85.5) after 28-32 months of use. The results demonstrate that the residual insecticide on the nets after up to 32 months of use was sufficient to kill the majority of susceptible *An. arabiensis* but not the wild caught mosquitoes. This suggests that there is some resistance to deltamethrin in wild mosquitoes in Ethiopia, enabling about half to survive in the bioassay tests.

## 990

### ADAPTIVE CHANGES IN AMINO ACID AND CODON BIASES OF THE MOSQUITO SODIUM CHANNEL IN THE EVOLUTION OF PERMETHRIN SELECTION

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Target site insensitivity resulting from point mutations within the voltage-gated sodium channel of the insect nervous system is known to be of primary importance in the development of resistance to pyrethroid insecticides. Here, we report a systematic analysis of nucleotide polymorphisms through the entire sodium channel cDNAs among susceptible, intermediately resistant parental, and highly resistant offspring mosquitoes *Culex quinquefasciatus* and the dynamics of the synonymous and nonsynonymous nucleotide composition of the mosquito sodium channel under insecticide selection pressure. Three nonsynonymous and 6 synonymous mutations were found in the *Culex* mosquito sodium channel. A comparative framework was used to examine adaptive changes in the patterns of amino acid and codon usage in the 3 *Culex* mosquito strains under permethrin selection pressure, revealing the frequency of both nonsynonymous and synonymous mutations in the mosquito sodium channel underwent a rapid population expansion following permethrin selection. This finding suggests permethrin selection is the prevailing selective force in the evolution of the amino acid and codon usage of the mosquito sodium channel, thus affecting the sensitivity of the sodium channel to insecticides and enabling the mosquito to survive.

## 991

### REDUCING SELECTION FOR RESISTANCE BY LOWERING PYRETHROID CONCENTRATION: THE EFFECTS OF AGE AND EXPOSURE HISTORY

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Malaria control campaigns usually involve widespread use of chemical insecticides to eliminate the mosquito vector. The fast-killing action of these compounds imposes strong selection for the evolution of resistance. It has recently been proposed that current delivery methods might be refined to target, rather than all potential vectors, only the immediately-

dangerous, infected mosquitoes and, therefore, we could reduce the pressure on the insects to evade chemical control as well as interrupt disease transmission. We investigated one possible selection-reducing manipulation of insecticide application, lowering the concentration to preferentially kill older mosquitoes. Specifically, we examined the effects of single and repeated exposure to low doses of the pyrethroid, permethrin, on survival of adult female *Anopheles* stephensi mosquitoes of different ages. Mosquitoes were exposed to permethrin at days 4, 8, 12 and 16 days post adult emergence using standard WHO resistance assay protocols and subsequently monitored for 24 days. Permethrin concentrations were less than half of the lowest recommended insecticide-treated net dose. We found that age at exposure had a greater impact on survival than number of times previously exposed, with older mosquitoes surviving less than younger ones at all concentrations. Though there was no increase in lethality with repeated exposure to low doses of permethrin, the overall reduction in survival, combined with a degree of age-discrimination, is consistent with the idea that doses of insecticide that do not kill 100% of all mosquitoes shortly after contact could still lead to effective disease control, if females are killed before they become old enough to contribute to transmission.

## 992

### THE EFFECT OF MOSQUITO AGE ON INSECTICIDE RESISTANCE STATUS

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Appropriate monitoring of insecticide resistance in mosquito vectors is a key component of vector control programs. A standard WHO protocol is routinely used to monitor insecticide resistance. The protocol uses 2-5 day old unfed mosquitoes exposed to filter papers impregnated with a discriminating dose of insecticide. There is some discussion as to whether mosquitoes become more susceptible as they age, although evidence for this is limited. This study investigated the effect of age on insecticide resistance status in a field collected strain of *Anopheles gambiae s.s.* from the Ivory Coast. Unfed females aged between 1 to 14 days were exposed to standard papers with deltamethrin, permethrin, DDT, bendiocarb and malathion following the standard procedure as described by the WHO. Using probit analysis the results of each tested age group were compared to show the time of exposure causing 50% and 90% knockdown. The 24 h post-exposure mortality of each different age group was also recorded. The laboratory standard susceptible strain KISUMU was used for control purposes. The significance of the results will be discussed in relation to current monitoring methods and the interpretation of data generated using this standard test.

## 993

### THE INCREASE IN THE UTILITY AND IMPORTANCE OF BIOMOLECULAR TECHNIQUES IN RESISTANCE MONITORING IN INSECT VECTORS

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The main problem associated with the onset of resistance is the failure to reduce vector populations and the potential for an increase in disease transmission. Monitoring will allow a timely change in strategy and various bioassays, biochemical and molecular methods exist that can be used to test and monitor resistance development. Identification of the resistance mechanisms involved gives an indication of which alternative compounds should be used. Molecular assays are an ideal complement to bioassays, and are especially useful to monitor trends in resistance gene frequency over time. Molecular assays can detect resistance at very low frequency, can indicate the presence of heterozygous individuals with recessive resistance genes that are not detected through bioassays and require fewer mosquitoes than bioassays. Their use is currently restricted to research labs since field test kits are still in development. Molecular techniques are now routinely used for identification of sibling species using the multiplex PCR assay and molecular M and S forms within the *Anopheles gambiae* complex using a restriction fragment length polymorphic (RFLP) PCR assay. The frequency of *kdr* alleles is detected using allele specific RT-PCR (Reverse Transcription Polymerase Chain Reaction); all of these assays can be carried out on phenotyped samples (survivors and non-survivors) of WHO susceptibility tests and departure from Hardy-Weinburg proportions can be examined. Microarrays have been developed and have demonstrated increases in RNA levels associated with oxidase (P450), Glutathione-S-Transferase (GST) and exterase (COE) activity in resistant mosquitoes. There is currently no field method to test for the presence of resistance associated P450s, COE and GSTs, other than bioassays with synergists. The development of these methods is essential to enable complete characterization of resistant populations. This will assist control programmes in making the most informed decision they can regarding appropriate insecticide choice and will contribute to the development and introduction of new insecticide classes needed for vector control.

## 994

### MORTALITY RATES AND INSECTICIDE RESISTANCE IDENTIFICATION FOR *ANOPHELES ALBUMANUS* (DIPTERA: CULICIDAE) IN NORTHERN PERU

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Mortality Rates and Insecticide Resistance Identification for *Anopheles albumanus* (Diptera: Culicidae) in Northern Peru. Insecticide resistance and cross-resistance in vector populations is a growing problem worldwide. Insecticide resistance by malaria vectors in Peru is largely unidentified. Peru possesses several malaria vectors to include *Anopheles albumanus*. An. albumanus populations were sampled using human landing collection within the department of Piura Peru and colonized to the F 42 generation. The CDC bottle assay method was used in this study. Approximately 20, three to six day old adult female mosquitoes were placed in 250ml Wheaton® bottles coated with the CDC's recommended diagnostic dosages for Anopheline mosquitoes. DDT, malathion, fenitrothion, etophenprox, permethrin, propoxur, lambda cyhalothrin, deltamethrin, alpha cypermethrin, cypermethrin, and bendiocarb were evaluated in this study. Eight replications were done for this study. Of the insecticides tested, mortality rates ranged from 100 percent down to 2%. Four insecticides had less than 90% mortality: deltamethrin (76%), alpha cypermethrin (60%), cypermethrin (19%), and bendiocarb (2%) indicating insecticide resistance to these compounds is present within this population of mosquitoes.

## 995

### DELIVERING INSECTICIDAL CRAB BAITS (ICB) AS A TOOL AGAINST *AEDES POLYNESIENSIS* BREEDING SITES IN LOW-ISLAND ENVIRONMENTS IN FRENCH POLYNESIA

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Burrows from the crab Cardisoma carnifex, found in atolls of French Polynesia, are one of the principal breeding sites for the mosquito Aedes polynesiensis, primary vector of Lymphatic Filariasis in the South Pacific. A novel method of mosquito control is the use of Insecticide-laiced Crab Baits (ICB). This technology is based upon the principal that crabs will harvest and deliver the treated bait into the crab burrow/mosquito breeding site. A semi-natural system mimicking crab burrows was developed in the laboratory allowing the assessment of both Bti (Mosquito

Bits®) and Methoprene (Altosid® pellets) on mosquito larvae mortality and the potential acute and long-term toxicity on non-target species Cardisoma carnifex under near-natural conditions. Both insecticides were selected for their environment friendly action. We evaluated their impact on juvenile crabs as well as on male and female adult crabs.

In the presence of alternative food, most ICB were taken up and eaten within one hour of their placement into the buckets. Exposure of mosquito larvae to BTI-treated "crab burrow" water resulted in high survival. Increasing the BTI dose 8-fold did not increase mortality. Methoprene bioassays by comparison generated very encouraging results with high mortality rates observed over a period of 4 months. These results indicate the potential suitability of Altosid®-based ICB formulation. Control of Ae. polynesiensis immatures developing in crab burrows was recorded without measurable adverse effects on the crab population. No mortality, acute or long-term toxic effects amongst juvenile or adult crabs was observed. Exposure to ICBs does not appear to impact crab development. All exposed juvenile crabs underwent at least one molt. ICBs impregnated with methoprene appeared to have no impact on non-target mosquito species (Culex pipiens and Toxorhynchites amboinensis) that utilized the semi-natural crab burrows as breeding sites during the experiment. If successful in the field, this ICB strategy could help suppressing the vector of LF in low islands of French Polynesia.

## 996

### CONTACT IRRITANCY RESPONSES IN *AEDES AEGYPTI* USING SUBLETHAL DOSES OF PYRETHROID CHEMICALS IN IQUITOS, PERU

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Dengue, transmitted by *Aedes aegypti*, is one of the most important viral diseases world-wide. Current methods for the reduction of disease transmission includes controlling adult vector populations using chemical applications at toxic doses; however, issues of insecticide resistance, adverse health effects and environmental concerns have driven the need to redesign our currently available tools. The use of chemicals at sublethal doses to modify vector behavior is one possible novel strategy. We have shown in previous studies that pyrethroids have irritant effects on *Ae. aegypti* at dosing levels below current field application rates (i.e., LD90). This irritant action induces movement of the insect away from a chemical source and can be exploited to promote exit from a space occupied by human hosts prior to biting thereby reducing the probability of disease transmission. This study, conducted in part within a larger research program to field-validate a Push-Pull strategy to reduce *Ae. aegypti* inside homes, quantified contact irritancy behavior of *Ae. aegypti* at sublethal doses for two pyrethroids insecticides: alpha-cypermethrin and deltamethrin using an experimental hut study design in Iquitos, Peru. Chemicals were applied to textile material at varying doses and surface area coverage. Laboratory reared female *Ae. aegypti* adults were released inside each experimental hut and exit movement patterns quantified every 30 min from 0600-1800h using interception traps to capture escaping mosquitoes. Results compared escape density rates over time among dose, surface area coverage and chemical variables. This information will be used to design the optimum contact irritant treatment scheme for experimental Push-Pull trials.

## 997

### IDENTIFICATION OF CANDIDATE GENES ASSOCIATED WITH DELTAMETHRIN RESISTANCE IN THE LATIN AMERICAN MALARIA VECTOR *ANOPHELES ALBIMANUS*

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Insecticide resistance is a wide spread phenomenom that undermines the efforts to control malaria. Identifying the genetic basis of insecticide resistance may lead to the development of novel tools for early detection of resistant populations. New tools include applications of transcriptomics and genomics that detect genes associated with resistant phenotypes. Although *Anopheles albimanus* is the predominant malaria vector in Central and South America, there have been few research efforts to study the response of this vector to insecticide exposure at the molecular level. Adult females of colonized *An. albimanus* were exposed to deltamethrin in an effort to detect transcripts associated with resistance. We used Suppressive Subtractive Hybridization (SSH) to identify differentially expressed genes in a two-pronged approach. First we identified genes over-expressed after exposure to a lethal dose 90 of 0.3 ug/ml deltamethrin (LD90). Additionally, we subjected a population to an LD90 during 18 generations, at which point unexposed females were taken from the colony under selection and compared with females from the unselected colony in a second SSH analysis. We expect that an overlap between the over-expressed transcripts in both experiments will reveal *An. albiman*us genes potentially associated with resistance to deltamethrin, such as P450 family members. We propose that this approach will increase the probability of selecting true candidates. Three transcripts were over-expressed in the colony under selection as compared with the unselected colony. These transcripts, as well as those differentially expressed between exposed and non exposed susceptible populations, are being sequenced to determine their identity. Further work will verify that over-expression of these transcripts is associated with resistance and may contribute to improving monitoring and detection techniques.

## 998

### INSECTICIDE SUSCEPTIBILITY OF *AEDES AEGYPTI* IN CARTAGENA (COLOMBIA)

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Dengue fever keeps an endemic behavior in Cartagena (Colombia), a location in the northern Caribbean coast of Colombia where insecticides have played an important role in actions towards the control of this disease during the last four decades. However, it is not known if the selective pressure on Aedes aegypti population in this location has induced some resistance to insecticides. Here, the susceptibility of Aedes aegypti in Cartagena was evaluated against organophosphorus, organochlorine and pyrethroid insecticides during a year (2009). Biological assays were carried out with adults (F2) and third-instare larva of A. aegypti collected in different urban districts including Los Alpes, Zaragocilla, and Pasacaballos. For adults, the CDC-Atlanta method was applied using diagnostic doses for malathion (100 µg/ml), fenitrothion (75 µg/ml), DDT (150 µg/ml) and lambdacyhalotrine (6,25 µg/ml). For larva, the World Health Organization method was applied, with a diagnostic dose of Temephos (0,012ppm). Each insecticide was tested three times, with four replicates each time, and a control with no insecticide was also included.

All three populations showed susceptibility to malathion, fenitrothion, deltametrine and propoxur, with a 100% mortality in all cases. Concerning to Lambdacyhalotrine (47-70% mortality) and Temephos (95 a 99%

mortality), variation was found in the susceptibility/resistance. In contrast, resistance was found in all three populations with 3-6% mortality In conclusion, our results suggest some degree of resistance to insecticides in three populations of A.aegyptis in Cartagena-Colombia. This might indicate a growing phenomenon of insecticides resistance in this location.

## 999

### ASSESSING THE EFFICACY OF DELTAMETHRIN-IMPREGNATED LETHAL TARGETS FOR THE CONTROL OF THE LYMPHATIC FILARIASIS VECTOR IN TAHITI, FRENCH POLYNESIA

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Lymphatic filariasis (LF) is one of the world's leading causes of disability. The ongoing Pacific Program to Eliminate Lymphatic Filariasis (PacELF) is based on a mass drug administration program to reduce human LF prevalence. A supplemental method to eliminate LF is the control of its main vector, Aedes polynesiensis, to reduce host-vector contact. The use of insecticide impregnated materials is showing great efficacy for the control of various mosquito-borne diseases, particularly malaria. We have been evaluating the efficacy of pyrethroid impregnated outdoor visual resting targets (Lethal Targets) to control this exophilic diurnal mosquito in the field. Following preliminary laboratory attractiveness tests of different colors, a navy blue 100% cotton fabric was selected. Effective impregnation with deltamethrin was verified using a standard WHO cone bioassay. Preliminary sampling was conducted in four different villages along the west coast of Tahiti to identify potential experimental field sites. Twice monthly collections were undertaken with BG Sentinel traps (Biogents, Regenwald) to derive baseline data on the vector populations and likely-use blocks (treatment and control) were selected in the village of Toahotu on the Tahiti peninsula. Permission was obtained from property owners to place a lethal target and to use a BG trap for weekly collections. Mosquito sampling during the month preceding deployment of the LTs is underway and will provide an estimate of the mosquito density prior to the treatment. A similar sampling regime will be used following placement of LTs to assess their overall impact on the mosquito population. Results of the ongoing trials will be presented.

## 1000

### LARVAL HABITAT SEGREGATION BETWEEN THE MOLECULAR FORMS OF THE AFRICAN MALARIA MOSQUITO, *ANOPHELES GAMBIAE* IN A RICE FIELD AREA OF BURKINA FASO, WEST AFRICA

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Disruptive selection acting on alternative eco-phenotypes can promote the evolution of reproductive isolation between populations, a process known as ecological speciation. In West Africa, lineage splitting between the M and S forms of the major Afro-tropical malaria mosquito, *Anopheles gambiae* is thought to be driven by ecological divergence, occurring mainly at the larval stage. Here, we will present evidences for habitat segregation between these two cryptic species in and around irrigated rice-fields located within the humid savannas background of western Burkina Faso, West Africa. Longitudinal sampling of adult mosquitoes emerging from a range of larval development sites was conducted from June to November 2009. Every other week, emergence traps were set up above larval development sites distributed along a 15km-long transect, from the heart of the rice-fields area into the surrounding savannas. In total, eighty larval development sites were georeferrenced and characterized (distance to the rice fields and to the nearest house, surface, depth, presence of standing vegetation, algae and/or debris, presence of predators and other culicine species, water origin, turbidity and general surrounding). A null model analysis revealed that the two molecular forms are non-randomly distributed (p=0.003). Canonical correspondence analysis was used to explore the spatial pattern of occurrence of the two sibling species and their relation to environmental variables. A major ecological gradient was extracted, in relation to the rice field perimeter (p=0.002). The M form was associated to larger breeding sites, which were mainly represented by rice field paddies. On the opposite, the S form was found to depend upon temporary, rain-filled breeding sites. These results support hypotheses about larval habitat segregation and confirm that both forms have different larval habitat requirement. Segregation appears clearly linked to anthropogenic permanent habitat and the community structure and diversity cascades they support.

## 1001

### HOUSEHOLD-LEVEL PREDICTORS OF *AEDES AEGYPTI* PRESENCE AND ABUNDANCE IN IQUITOS, PERU: IMPLICATIONS FOR DENGUE CONTROL

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Understanding what factors affect *Aedes aegypti* presence and abundance in/around houses enhances opportunities to target high-risk sites for mosquito control efforts. Using extensive *Ae. aegypti* surveillance data collected throughout Iquitos, Peru during 1999 through 2002, we evaluated associations between household characteristics and presence of adult mosquitoes. Houses with at least one adult *Ae. aegypti* captured with backpack aspiration (cases) were compared with mosquito-free houses (controls). Matching of case and control houses for space (<100 m distance) and time (same day) was performed to eliminate possible confounding from non-house factors and changes in mosquito abundance over time. Vegetation coverage surrounding houses was estimated from NDVI values (Landsat satellite images). Adjusted odds ratios were calculated using conditional logistic regression. Significantly more houses with adult *Ae. aegypti* had open soffit and room partitions (OR = 1.46, p = <0.0001), more manually filled containers (OR1 Container Increase = 1.02, p = <0.0001), more naturally rain filled containers (OR1 Container Increase = 1.05, p = <0.0001), more containers filled via roof runoff (OR1 Container Increase = 1.12, p = <0.0001), more residents (OR1 Person Increase = 1.03, p = <0.0001), and greater vegetation (OR = 1.05, p = <0.0016). These results demonstrate a complex pattern of household-level biophysical and social factors that are associated with transmission risk in this region of endemic dengue, and suggest contexts where interventions might be more effective.

## 1002

### EFFECTS OF BACTERIAL GROWTH ON THE HATCHING OF *AEDES AEGYPTI* EGGS

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It is well established that eggs of *Aedes aegypti* will hatch when the water in which they are submerged is contaminated by microbes. Effects of bacteria on egg hatch is commonly thought to result from a decline in dissolved oxygen (DO) concentration during microbial growth. Notably, there have been no studies to establish effects of bacterial age and other factors associated with bacterial growth on egg hatching. We hypothesized that metabolites associated with bacteria growth or bacteria themselves would stimulate hatching. To test these hypotheses, we exposed eggs of *Ae. aegypti* to bacterial cultures of varying age and assessed subsequent percentage hatch over a time course of one to 4-h. The bacterial cultures were comprised of a mix of 14 species that were originally cultured from an experimental plant infusion constructed from senescent leaves of the bamboo plant *Arundinaria gigantea*. Levels of DO were measured concurrently. In 24-h old stationary phase cultures (cell density = 3.5X109 CFU/ mL), 95% of eggs hatched in 1-h, whereas for 8-d old dead phase cultures (5.9X107 CFU/ mL) only 9% of the eggs hatched in 1-h. DO in the 24-h and 8-d old cultures averaged 0.81 mg/L and 2.8 mg/L, respectively. Surprisingly, 92% of eggs hatched within 1-h after exposure to a 6-h old log phase bacterial culture (7.4 X106 CFU/ mL) at 5.0 mg/L DO. Additionally, an average of 93% of eggs exposed to bacteria cells suspended in 0.85% NaCl solution hatched within 4-h at an average DO of 7.6 mg/L. In comparison, from 0 to 3% of eggs hatched in deionized water or the saline solution even after an exposure period of 5-d. These results suggest that the hatch of *Ae. aegypti* eggs is mediated by bacteria and/or bacteria-associated factors irrespective of DO concentration.

## 1003

### FEEDING PATTERNS AND ARBOVIRUS DETECTION IN *CULEX (MELANOCONION) TAENIOPUS* (DIPTERA: CULICIDAE) FROM GUATEMALA

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Several mosquito-borne arboviruses including members of the *Flavivirus*, *Alphavirus* and *Orthobunyavirus* genera are important zoonotic agents that may cause febrile and encephalitic illness in vertebrate hosts. *Culex (Melanoconion) taeniopus* mosquitoes were associated with transmission of *Venezuelan equine encephalitis virus* (VEEV; alphavirus) and *Nepuyo virus* (bunyavirus) in Guatemala in the late seventies, and have been shown in experimental studies to be efficient vectors of VEEV. During an arbovirus ecology study conducted in the department of Izabal, Guatemala from 2007-2009, we collected 5171 *Cx. taeniopus* using CO2-baited CDC light and gravid traps. To better define the role of *Cx. taeniopus* as a vector of arboviruses, engorged mosquitoes were removed from the collections for blood meal host determination by PCR tests using mitochondrial *Cyt b* and *COI* primers followed by amplicon sequencing. Species-specific DNA sequences were determined from comparison to known sequences in GenBank and the Barcode of Life database (BOLD). All mosquitoes were tested (in pools or individually) by RT-PCR and Vero plaque assays to detect arboviruses. As a result, viruses of all three genera indicated above were detected. Vertebrate hosts were identified from 262 blood meals (85% of 308 engorged mosquitoes tested). Mammals and birds comprised 28% and 31%, respectively, of the identified blood meals. Dog and cow were among the most common mammalian hosts and chicken was the most common avian host. Our results indicate that *Cx. taeniopus* carries several arboviruses in Guatemala and would probably serve a bridging function between sylvatic vertebrates and peridomestic hosts including people and their domestic animals.

## 1004

### NATURAL INFECTION RATES BY *PLASMODIUM* SPP. OF ANTHROPOPHILIC ANOPHELINES FROM LOCALITIES OF NORTHWESTERN COLOMBIA

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In Colombia, malaria remains an important public health problem. The assessment of natural infection by *Plasmodium* spp. in anopheline mosquitoes is an important aspect to determine the role of different *Anopheles* species in malaria transmission. We evaluated natural infection rates (IR) by *Plasmodium* spp. in 6,463 anophelines collected using human landing catches. The collections were realized between January and November 2009 in six localities of two malaria regions of Colombia: Urabá - Bajo Cauca-Alto Sinú and the Pacific Coast. Nine *Anopheles* species were detected in three subgenera: *An. nuneztovari* s.l. (58.42%), *An. darlingi* (36.80%), *An. albitarsis* s.l. (1.73%), *An. albimanus* (1.70%), *An. triannulatus* s.l. (1.12%), and *An. punctimacula, An. neivai, An. pseudopunctipennis* and *An. neomaculipalpus* at <0.25%. ELISA and molecular confirmation by nested PCR showed that only two species were naturally infected by *Plasmodium* spp.: *An. nuneztovari* s.l. and *An. darlingi*. Two *An. nuneztovari* s.l. from Buenaventura-Valle del Cauca were found naturally infected with *Plasmodium vivax* VK247 (IR=1.8%), and three specimens from Puerto Libertador-Córdoba were infected, one with *P. falciparum* (IR=0.05%) and two with *P. vivax* VK210 (IR=0.1%). One specimen of El Bagre-Antioquia was infected with *P. vivax* VK247 (IR=0.62%). The *An. darlingi* infected with *P. falciparum* (IR=0.09%) was from Vigía del Fuerte-Antioquia and the other with *P. vivax* VK210 (IR=0.28%) from El Bagre-Antioquia. These findings suggest that *An. nuneztovari* s.l. and *An. darlingi*, two major malaria Colombian vectors continue to play an important role in malaria transmission in these regions. These results constitute the most recent known reports of anophelines naturally infected by *Plasmodium* spp. in these localities and contribute to the understanding of the species involved in the transmission in these regions, information that is useful for the design of selective vector control strategies.

## 1005

### VECTOR BIOLOGY OF ANOPHELINE MOSQUITOES IN LA CAPILLA-EL BAGRE, ANTIOQUIA, COLOMBIA

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Antioquia is one of the departments most affected by malaria transmission in Colombia, and El Bagre (Ant) was the municipality reporting the highest number of cases in 2009 (8,909). Characterization of the bionomic aspects of anophelines involved in malaria outbreaks provides important information for targeted control strategies. We determined the abundance, biting behavior and entomological inoculation rate-EIR of the

anopheline species present in La Capilla, El Bagre from January-December 2009. Six-day collections, every three months, were conducted using human landing catches, outdoors and indoors from 18:00-0:00 h with a one-day collection from 18:00-06:00 h. A total of 2,459 anophelines belonging to six species were identified, *Anopheles darlingi* (49%), *An. nuneztovari* s.l. (42%), *An. albitarsis* s.l. (5%), *An. triannulatus* s.l. (3.6%), *An. punctimacula* (0.24%) and *An. pseudopunctipennis* (0.12%). Mostly all species were more abundant at the onset and the end of the rainy season. Only *An. nuneztovari* and *An. darlingi* were infected by *Plasmodium vivax*. *An. nuneztovari* presented a marked endophagic behavior and *An. darlingi* had variable behavior. There was a significant difference between the mean numbers of *An. nuneztovari* indoors and outdoors ($t$=4.53, $P$≤0.001, $n$=180), while the difference was not significant for *An. darlingi* ($t$= -0.18, $P$≤0.85, $n$=180). Both species were active throughout the night; *An. nuneztovari* presented three main peaks at 21:00-22:00, 23:00-00:00 and 03:00-04:00 and *An. darlingi* showed higher activity at 19:00-00:00. Their EIR values did not differ significantly. The results demonstrate that *An. nuneztovari* and *An. darlingi* have an important role in malaria transmission in this locality. Even though less abundant species collected were not infected, further studies will help to clarify their potential roles in malaria transmission at the local level, since all the species identified have been incriminated as vectors in other regions.

## 1006

### VEGETATION CHARACTERISTICS AND WEST NILE VIRUS TRANSMISSION POTENTIAL IN SUBURBAN NEIGHBORHOODS

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Transmission of West Nile virus (WNV) occurs primarily among vector mosquitoes and avian reservoir hosts. Prevalence of infection in mosquitoes, birds or other hosts varies temporally and spatially with virus quantity and replication rate, host-seeking behavior of mosquitoes, and the proximity of infectious vectors and hosts. Vegetation affects transmission as forage and cover for vectors and hosts and mediates the effects of temperature, humidity and rainfall. We examined how vegetation measured in several ways provides innovative and scale-appropriate characterization of suburban landscapes to gain insight into transmission risk. Risk related to vegetation is assessed as the association of landscape characteristics with abundance of hosts and vectors and WNV infection. We interpret those in the context of infection risk in a region of suburban Chicago, Illinois, where West Nile virus has been observed since 2001. Vegetation characteristics included size, abundance, species, and spatial distribution and arrangement. We measured characteristics of microhabitats by combining methods involving digital processing of remotely sensed data, visual interpretation of image features, and analysis of direct observations. We created landscape metrics from these observations with FRAGSATS software and other spatial techniques. Transmission risk was estimated from field observations of infection and density of vectors and avian hosts from suburban neighborhoods, including numbers of *Culex* mosquitoes from light traps, WNV infection rates in mosquito pools and sampled birds, and locations of American robins (*Turdus migratorius)*, a species of interest in WNV transmission in the Chicago area. Multivariate statistics were used to test the statistical significance of relationships. We present novel measures of regional urban vegetation, an analysis relating these to the habitats of *Culex* species mosquitoes and American Robins, and an interpretation in light of risk of infection. Mosquito infection was higher in sites with taller and larger trees, while mosquito abundance was associated with more canopy cover, density of stems, presence of contiguous vegetation, and fewer trees in the Rosaceae. These methods to characterize vegetation are useful to elucidate transmission dynamics of WNV at a fine spatial scale and may be applied to other peri-urban arboviruses, such as Dengue virus and Ross River virus.

## 1007

### TRAPPING STUDIES WITH THE MALARIA VECTOR *ANOPHELES DARLINGI* IN SURINAME AND THE RELATION WITH BITING PREFERENCES

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The effectiveness of CO2-baited and human-baited mosquito traps at sampling An. darlingi mosquitoes was evaluated and compared to human landing collections (HLC). Biting preferences of this mosquito on a human host were studied and related to the trapping data. Traps used were the CDC Miniature Light Trap (with and without light), the BG Sentinel Mosquito Trap (without BG Lure), the Mosquito Magnet ® Liberty Plus Mosquito Trap (MM-Plus) (without octenol) and a custom design trap. Carbon dioxide or humans were used as bait. The number of An. darlingi collected was greater with the HLC, than with all other collection methods. None of the traps correlated with the HLC in number of An darlingi captured over time. Of the traps evaluated the BG Sentinel Mosquito Trap with CO2 or human bait and the MM-Plus, proved most efficient in collecting An. darlingi. In the field study on An. darlingi biting preferences the females showed directional biting behaviour ($p<0.001$) with a majority of females (93.3 %) biting the (lower) leg and feet region when confronted with a human host sitting down. Higher efficiency of the closer-to-the-ground collecting MM-Plus and BG Sentinel Mosquito Trap may be a result of this biting preference of the vector.

## 1008

### ENVIRONMENTAL AND SOCIOECONOMIC FACTORS LIMITING THE DISTRIBUTION OF *AEDES AEGYPTI* IN THE VENEZUELAN ANDES

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Dengue fever is a serious acute illness caused by infection with one of four viral strains. It is spread through the bites of infected mosquitoes, the primary vector in Venezuela being *Aedes aegypti*. The objective of this study was to determine whether temperature and other environmental factors limit the distribution of *Ae. aegypti* in high altitude areas, while controlling for socioeconomic determinants. During the summer of 2009, 24 randomly selected sites distributed across three cities (El Vigia, Ejido and Merida) ranging in altitude from 70 to 1950m were surveyed for mosquito breeding habitats in and around the homes. Occupant interviews were conducted to assess socioeconomic status and access to relevant infrastructure, such as trash collection and water services. For the highest and largest city (Merida), environmental variables were derived from an Advanced Spaceborne Thermal Emission and Reflection Radiometer (ASTER) satellite image. These included land surface temperature, elevation, the mean and standard deviations for each ASTER spectral band, the Normalized Difference Vegetation Index (NDVI) and the Normalized Difference Built Index (NDBI). El Vigia, the town at the lowest elevation, had a higher proportion of positive containers (17.7%) than Merida and Ejido (10.5% and 10.7%, respectively). Water holding containers were more common in El Vigia, while flower pots and vases - linked to better socioeconomic conditions, were more frequent in the

other two towns. In Merida, the proportion of positive containers was higher in warmer and less vegetated areas (lower NDVI). Mean house size and number of inhabitants were also positive predictors, while access to water and trash services was not found to be predictive, in contrast with previous studies in coastal Venezuela. In conclusion, environmental factors derived from remotely sensed data show a closer association with the distribution of *Ae. aegypti* larvae in high altitude areas than socioeconomic factors found to be strongly predictive in other dengue endemic areas.

## 1009

### *MANSONIA* SPECIES AS POTENTIAL VECTORS OF LYMPHATIC FILARIASIS IN GHANA

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*Wuchereria bancrofti*, the causative agent of lymphatic filariasis (LF) is transmitted by mosquito species belonging to *Anopheles, Aedes, Culex* and *Mansonia*. In East Africa, *Anopheles*, *Culex* and *Mansonia* species are known vectors but in West Africa including Ghana only *Anopheles* species are reported vectors. *Anopheles gambiae* s.l and *An. funestus* are the main vectors with An. pharoensis playing a minor role. The mosquito species involved in the transmission of LF is important to achieve the goal of elimination using only mass drug administration (MDA) with ivermectin/ DEC and albendazole. It is recognized that it may be difficult to eliminate LF when the vectors are culicines because they exhibit "limitation" while anophelines show "facilitation". Collections of mosquitoes in LF endemic areas in Ghana have shown large numbers of *Culex* and *Mansonia* species and while it has been established that *Culex* does not transmit the disease in Ghana, the status of *Mansonia* is not known. Recent data from Ghana has shown that after 6 rounds of annual MDA there is still a high prevalence of the disease in some areas where An. melas, *An. gambiae* s.s., *Mansonia* and *Culex* species are the main biting mosquitoes. It has therefore become necessary to determine whether *Mansonia* species play a role in the transmission of LF in these areas. Indoor mosquitoes were collected once a month for three months using pyrethrum spray catches between the hours of 0500-0800 GMT in six communities (Atabadze, Anyinase, Bandor, Epoano, Ponkrom and Sanka) in the KEEA district of the Central Region of Ghana. A total of 824 mosquitoes composed of 500 *Anopheles* species, 240 *Mansonia* species and 84 *Culex* species were caught, dissected and examined for the presence of *W. bancrofti*. Four infected *Mansonia africanus* were found at Sanka with all developmental stages (L1, L2 and L3) of *W. bancrofti*, one *M. africanus* with L2 at Anyinase and one *An. gambiae* s.s with an L3 at Epoano. This is the first report indicating *Mansonia* species as possible vectors of LF in Ghana and West Africa.

## 1010

### POTENTIAL YELLOW FEVER VECTORS AND THEIR ECOLOGY IN THE PAGA COMMUNITY IN THE KASSENA-NANKANA DISTRICT, UPPER EAST REGION OF GHANA

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Yellow fever (YF) is an acute, infectious, haemorrhagic viral disease with significant public health impact in the tropics transmitted mainly by Aedes mosquitoes which also transmit dengue haemorrhagic fever. Epidemics of yellow fever have previously occurred in Ghana particularly from the 1970s to the 1990s. Recently there have been YF outbreaks in Burkina-Faso which shares borders with Ghana and unconfirmed reports in certain localities in Ghana. Unfortunately, the last detailed work on the vectors in Ghana was published in 1975. There is therefore the need to update the data on yellow fever vectors in Ghana especially within communities along the Ghana-Burkina Faso border. We studied potential yellow fever vectors and their ecological characteristics in Paga at the Ghana-Burkina Faso border in the Kassena-Nankana District (KND). Mosquitoes were collected from 40 households in the Paga community from human landing catches, larval collections and ovitraps for two months each in the rainy and dry seasons. A total of 1197 mosquitoes were collected and morphologically identified. Of these, 609 (50.9%) were *Aedes aegypti*, 423 (35.3%) were *Ae. vittatus* while the remaining 13.8% consisted of *Ae. africanus, Ae. simpsoni*, *Anopheles gambiae*, *Culex* and *Mansonia* species. *Ae. aegypti*, *Ae. vittatus, Ae. africanus* and *Ae. simpsoni* were found breeding in different water holding containers. *Ae. aegypti* larvae were found prevalant in earthenware pots (65.6%), which is the most common water holding container in the area followed by car tyres (34.4%) while Ae. vittatus bred mostly in rock pools (98.6 %). Most of the breeding occurred in the rainy season. Also *Ae. aegypti* and *Ae. vittatus* were the most common biting mosquitoes (50.9% vrs 35.3%) with *Ae. aegypti* biting mostly outdoors (59.2%, N=201) in the rainy season. A high proportion of *Ae. aegypti* (45.3%) was found laying eggs in the ovitraps during the rainy season compared to 4.1% during the dry season. These observations have implication for yellow fever transmission in the community.

## 1011

### FIRST REPORTED HUMAN CASE OF JAMESTOWN CANYON VIRUS INFECTION IN MONTANA, 2009

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Jamestown Canyon virus (JCV) is a mosquito-borne zoonotic pathogen belonging to the California (CAL) group viruses and the Bunyaviridae Family. Although JCV is widely distributed and has been detected in >20 mosquito species throughout temperate North America, reports of human JCV infections in the U.S. are rare, and generally confined to the Midwest and eastern states. We report the first detected case of human JCV infection in Montana. On May 26, 2009, a 51-year-old male resident of MT with no travel history presented to the emergency room (ER) with severe acute frontal headache, fever, dizziness, and left unilateral numbness and tingling. Initial testing in the ER revealed normal blood chemistries, electrocardiogram, CT scan, and MRI, and was released. Six days post-onset, the patient visited his primary care physician exhibiting headache, muscle pain, and muscle weakness consistent with encephalitis. Acute- and convalescent-phase sera were tested for West Nile (WN), Saint Louis Encephalitis (SLE), LaCrosse (LAC), and JC viruses. Positive IgM and IgG enzyme linked immunosorbent assays (ELISA), static neutralization titres, and high WN virus IgG avidity results indicated a previous WN virus infection. However, plaque reduction neutralization tests (PRNT) revealed a four-fold rise between samples in JCV titers while an acute-phase sample IgM ELISA test result was equivocal for LAC virus antibody using a LAC antigen preparation. Because CAL group virus antibody cross reactivity may occur to various degrees in IgM ELISAs that utilize only one kind of CAL group antigen, IgM ELISA assays incorporating JCV antigen were subsequently performed. Positive JCV IgM ELISA values were documented for the patient's sera, and the presence of JCV specific IgM and the observed diagnostic rise JC specific antibody by PRNT confirmed JCV infection. This finding may represent a previously unrecognized JCV focus in MT and a need for MT clinicians to consider JCV infection in differential diagnoses for patients with unexplained febrile or encephalitic illness. As well, incorporation of both LAC and JC virus antigens in screening ELISAs should be considered when performing CAL serogroup virus serology.

## 1012

### RE-EVALUATING THE LINK BETWEEN MALARIA AND CLIMATE

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Malaria transmission is strongly influenced by environmental temperature but the biological drivers remain poorly quantified. Most studies analyzing malaria-temperature relations, including those investigating malaria risk and the possible impacts of climate change, are based on mean temperatures and extrapolate from functions determined under unrealistic laboratory conditions. Here we show how the influence of temperature fluctuations and extreme events can be as, or more important than changes in mean conditions for malaria transmission. We investigated the effects of mean temperature and temperature fluctuation on key aspects of mosquito and parasite life history using a combination of novel empirical and theoretical approaches. We find that, in general, temperature fluctuation reduces the impact of increasing mean temperatures. Specifically, we show that diurnal temperature fluctuation around warmer mean temperatures slows processes such as larval development and parasite incubation, whereas fluctuation around cooler mean temperatures speeds up these processes, compared with constant temperatures. These effects suggest that by ignoring fluctuation, we may currently be overestimating malaria risk in warmer environments, and underestimating risk in cooler environments. This role of daily variation has rarely been considered in the dynamics and distribution of malaria. If we are to optimize control efforts and develop appropriate adaptation or mitigation strategies for future climates, we need to incorporate into predictive models the effects of daily temperature variation and how that variation is altered by climate change.

## 1013

### FLORAL-BASED ATTRACTION OF *CULEX* MOSQUITOES

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Feeding on plant sugars and nectar is essential for providing energy for flight and enhanced longevity of *Culex* mosquitoes. Relatively little is known about the cues used for location of plant sugars and nectars and this study focused on volatile cues associated with flowers. Discovery of attractants associated with flowers may provide the basis for development of novel surveillance methods for *Culex* mosquitoes. Flowers of several common plant species of plants in north-central Florida were discovered to effectively attract mosquitoes of several *Culex* species in olfactometer assays. Day-old males and females with no prior exposure to sugar or flowers responded equally well to flowers. Responses of day-old females and 7-10 day old mosquitoes (previously sugar-fed but with no flower exposure) were similar. These responses increased with duration of sugar-starvation. Volatile compounds from flowers were collected by grab sampling of headspace with vacuum silonite-lined bottles and by solvent (hexane, dichloromethane) elution from solid phase adsorbents (i.e. Porapak Q, Hay-Sep). Compounds in these samples were identified by GC/MS using 3-stage trap and purge or direct injection. Several identified compounds were effective in attraction of sugar-starved day-old mosquitoes when evaluated in an olfactometer. The addition of flowers or chemicals effective in the olfactometer enhanced collections of *Culex quinquefasciatus* and *Cx. nigripalpus* in MMX traps under field conditions.

## 1014

### THE IMPACT OF HOUSE SCREENING ON BITING BEHAVIOR OF *ANOPHELES* SPECIES AND TRANSMISSION OF LYMPHATIC FILARIASIS AND MALARIA IN GOMOA MAMPONG, A RURAL COMMUNITY IN GHANA

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Malaria and lymphatic filariasis (LF) account for the majority of mortalities and morbidities due to parasitic infections worldwide. While chemotherapy is one of the strategies for malaria control, it is the mainstay for LF elimination. Vector control using bednets which has been an integral part of malaria control has been intensified recently with the inclusion of indoor residual spraying (IRS) with insecticides. These vector control interventions are known to impact LF transmission especially in areas where the same *Anopheles* species transmit both diseases. The interventions target indoor biting and/or resting mosquitoes thus leaving outdoor biting mosquitoes mostly unaffected. There is therefore a critical need to determine the role of outdoor biting mosquitoes in the transmission of malaria and LF. We examined the effect of a vector control intervention on *Anopheles* biting density and infection with *Plasmodium* species and *W. bancrofti* at Gomoa Mampong in the Central Region, Ghana where mass drug administration with ivermectin and albendazole against LF has been going on for 7 years. Mosquitoes were collected indoor and outdoor from two houses one of which has been screened against indoor biting mosquitoes and the other without any intervention. Sampling was done for five months using human landing collection and the mosquitoes identified morphologically and with PCR-RFLP. *Anopheles* species were dissected for *W. bancrofti* and examined with ELISA for infections with *Plasmodium* species. A total of 4563 *Anopheles* species were collected of which the house with screens had 2339 (51.3%) outdoors and only 80 (1.8%) indoors. Comparative biting density for the house without screens were 1575 (34.5%) outdoors and 569 (12.5%) indoors. Thus, screening appears to have shifted the biting from predominantly indoor to outdoors. All the mosquitoes dissected were negative for *W. bancrofti* reflecting the impact of the MDA in the area. None of the 334 indoor biting *Anopheles* was infected with *Plasmodium* while those collected outdoors gave a sprozoite rate of 0.42% (2/475).

## 1015

### ROLE OF AN INVASION-INDUCED HEME PEROXIDASE FROM *ANOPHELES GAMBIAE* DURING *PLASMODIUM* DEVELOPMENT

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Invasion of *Anopheles gambiae* midgut epithelium by *Plasmodium berghei* ookinetes causes severe damage to the invaded cells which ultimately leads to apoptosis. Invaded cells induce nitric oxide synthase (NOS) expression which catalyses the formation of nitric oxide (NO). This highly reactive NO is quickly converted into nitrite (NO2-) and triggers an extensive protein nitration in ookinete-invaded cells. Our group has demonstrated that there is a lapse in time between the process of protein nitration in invaded cells and the induction of NOS. Also, the process seems to require peroxidase activity capable of catalyzing tyrosine nitration in the presence of nitrite and hydrogen peroxide, as reported previously. A search of the *An. gambiae* genome revealed the presence of 16 different peroxidase genes; ookinete invasion of midgut cells induces the expression of 5 of them. Studies using double-stranded (ds) RNA-mediated knock down of these candidates in susceptible G3 mosquitos showed that only one of them, a heme peroxidase named HPX2 (AgHPX2), resulted in a significant increase in *P. berghei* oocyst numbers compared to dsLacZ-injected controls. Using colorimetric assay with a peroxidase-specific substrate, TMB, in the presence of hydrogen peroxide, we observed

that AgHPX2 silencing decreased the peroxidase activity induced by *Plasmodium* invasion in midguts. A corresponding decrease in tyrosine nitration levels occurred when AgHPX2 is silenced in infected midguts. These findings indicate that AgHPX2 is responsible for *Plasmodium* induced peroxidase activity in the midgut and it is an important part of the protein nitration process observed in ookinete-invaded cells. Immunofluorescence microscopic studies are underway to localize AgHPX2 in midgut cells.

## 1016

### LIFE-HISTORY OF *AEDES ALBOPICTUS* ADULTS UNDER DIAPAUSE CONDITIONS

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Vector-born infectious diseases are experiencing resurgence in temperate regions of the World, even in developed countries. The weather in such regions often includes a period of cold weather deadly to most adult or larvae mosquitoes. *Aedes albopictus* the Asian tiger mosquito is an important vector of dengue and yellow fever and most recently of chikungunya fever. This species of mosquito is thought to be originally tropical but it has adapted to temperate conditions by producing diapausing eggs and has expanded widely worldwide both in tropical and temperate regions. Diapausing eggs are cold tolerant and dormant and are produced by adult females after they experience levels of daylight below a locally adapted critical photoperiod. We quantified the life-history traits of adults reared under summer and fall light conditions, and compared female oviposition behavior in laboratory choice tests. We compared the effects of food and access to oviposition sites on the number of eggs deposited. We also examined the effect of presence of eggs on oviposition behavior and on egg retention by females under both diapause and non-diapause conditions. Our objective is to develop a predictive model of the both the rise and fall of abundance of *Ae. albopictus* during the active season and to identify putative costs and weaknesses of the adaptation to temperate climates of this and other critical nuisance mosquitoes and disease vectors.

## 1017

### ESTABLISHMENT OF POPULATION MALARIA SURVEILLANCE IN KAGERA REGION, TANZANIA, BY ROUTINE TESTING FOR PARASITEMIA AT TIME OF MEASLES VACCINATION AND FIRST ANTENATAL CARE ATTENDANCE TO EVALUATE IMPACT OF MALARIA INTERVENTION

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As malaria interventions are scaled up, urgency exists to provide data to demonstrate impact on the disease. The most accepted impact measures are from large, infrequent, nationally representative population surveys. Programmatic decisions need more frequently measured impact data from lower administrative levels. To evaluate the real-time impact of Indoor Residual Spraying (IRS) in Kagera Region (40,838 km$^2$) in north-western Tanzania, we introduced a surveillance system to test for parasitemia in children and pregnant women attending reproductive and child health (RCH) clinics. All children attending for regular measles vaccinations (9 months of age) and all pregnant women attending their first antenatal care visit were tested using routinely available HRP2-based Rapid Diagnostic Tests (RDTs) in 17 health centers (2$^{nd}$ level facilities) located in IRS (operations begun within past two years) and non-IRS areas of Kagera. The RCH clients came from a catchment of 71 villages. In Jun-Oct 2009, 77% of children and pregnant women attending RCH clinics were tested with an RDT. Among clients tested, 3,884 and 2,916 came from IRS or non-IRS areas, respectively. The overall positivity rate was 2.8% (95% CI: 2.5% to 3.5%) and 9.6% (95% CI: 8.9% to 11.1%) in IRS and non-IRS villages, respectively. No parasitaemia was detected in 29% of IRS villages (n=28) compared with 12% of non-IRS villages (n=43). Low positivity rates (<5%) were detected in 54% of IRS villages compared to 9% of non-IRS villages. In 49% of non-IRS villages the positivity rate exceeded 10% compared to 4% of IRS villages. In conclusion, children receiving measles immunization and pregnant women attending RCH clinics in Kagera represent a readily accessible population for directly monitoring the impact of various malaria interventions. This system is providing data over time and will allow us to evaluate the impact of single or multiple vector control strategies, particularly IRS alone or combined with insecticide treated bednets (recently distributed to all children <5 years).

## 1018

### MOSQUITO FEEDING AND POST-BLOOD MEAL FLIGHT BEHAVIOR IN BERNALILLO COUNTY, NEW MEXICO: IMPLICATIONS FOR TRANSMISSION AND CONTROL OF WEST NILE VIRUS

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West Nile virus (WNV) causes annual mortality and morbidity in animals, including humans, across North America. In Bernalillo County, New Mexico, potential mosquito vectors have been identified (*Culex quinquefasciatus, C. tarsalis,* and *Aedes vexans*), but little is known about which species is most important in maintaining the natural, sylvatic transmission cycle or in transmitting WNV to mammals of interest. Since 2004, mosquitoes have been collected weekly, identified to species, and tested for WNV. Since 2006, mosquito blood meals have been analyzed to determine which, if any, species is most likely to feed on known avian reservoir hosts or on mammals of interest. Of the potential mosquito vectors, *C. quinquefasciatus* derived >85% of its blood meals from avian hosts; of these >20% were American robins, known to be competent WNV reservoir hosts. *Aedes vexans* fed almost exclusively on mammals (>95%), while *C. tarsalis* fed on mammals and birds (62% and 38%, respectively). Bernalillo County has a zoo, and because zoo animals are kept in enclosures, mosquito flight distance can be estimated, post-feeding from enclosure to trap site. Analysis indicated that mosquito species generally took blood meals less than 140 meters from the trap site. For the 2004-2008 seasons, prevalence of WNV was higher in *C. quinquefasciatus* than *C. tarsalis* and *A.vexans*, 6.56%, 3.99%, and 0.83%, of pools tested respectively. Preliminary data suggest that *C. quinquefasciatus* with its strong preference for avian hosts and high infection rate it is important in maintaining the sylvatic cycle of the virus as well as in transmission to mammals.

## 1019

### *AEDES AEGYPTI* INFECTION BARRIER MAY LIMIT URBAN TRANSMISSION OF MAYARO VIRUS IN IQUITOS, PERU

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Mayaro virus (MAYV) is a forest-associated, mosquito-borne alphavirus circulating in South America. Although current evidence suggests a sylvatic transmission cycle maintained by canopy-dwelling mosquitoes such as *Haemagogus janthinomys*, the ability of MAYV to emerge and to be transmitted by an urban vector could have significant implications for public health. In Iquitos, a city of approximately 350,000 people in the Amazon region of Peru, an average of six cases of Mayaro fever are identified annually through a clinic-based surveillance network established

by the U.S. Naval Medical Research Center Detachment-Lima. Due to the identification of Mayaro fever cases in Iquitos clinics, and because *Aedes aegypti* is well-established in the city, we assessed the potential for *Ae. aegypti* to serve as an urban vector for MAYV. To investigate laboratory vector competence, infection, dissemination and transmission rates of MAYV in an F1 generation of *Ae. aegypti* from Iquitos, Peru were determined following artificial and viremic mouse feedings. Infection rates ranged from 0% (0/22) at a blood meal titer of 1.3 x 105 pfu/ml of MAYV to 84% (31/37) at 2.2 x 107 pfu/ml. The rate of dissemination varied from 60% to 100% of infected mosquitoes, independent of dose. Importantly, transmission of MAYV from 70% (21/30) of infected mosquitoes was demonstrated by capillary tube feeding and by bite on suckling mice. These data suggest that a midgut infection barrier, rather than midgut escape or salivary gland barriers, may be a limiting factor to *Ae. aegypti* serving as a vector in Iquitos. Human viremias may be below the threshold of infectivity for MAYV in this strain of A*e. aegypti*, explaining the limited number of cases of MAYV seen in urban Iquitos and the strong rural bias to human transmission in the region.

## 1020

### ENGINEERED STERILE MOSQUITOES FOR DENGUE CONTROL - FROM LAB TO FIELD

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Recent advances in insect genetic engineering have opened new possibilities for the control of mosquitoes and hence of mosquito-borne diseases. Oxitec has developed strains of *Aedes aegypti* and *Aedes albopictus* which are homozygous for one or more dominant lethal genes and are "genetically sterile" unless provided with the repressor molecule tetracycline in the diet. Use of such strains for mosquito control, a method known as RIDL, is based on the Sterile Insect Technique (SIT) which has been used successfully for the suppression or local elimination of several insect species in agriculture. Sterile male mosquitoes are released continually over a wide area to mate with the target pest population; no progeny result from these matings and the target population declines. Mathematical modeling indicates RIDL-SIT would be effective against *Aedes* mosquitoes. The first engineered strains with the necessary genetic properties ('RIDL strains') have been successfully tested in confined conditions for mating competitiveness with wild-type mosquitoes, suppression and a range of life history and behavioural traits in a range of locations and conditions. Preparations are underway for field trials to demonstrate suppression of wild populations. This presentation will summarise the results of experiments to date and discuss the options for testing and programmatic use of such technology.

## 1021

### DIFFERENCES IN THE CLINICAL FEATURES ACCORDING TO GENOTYPES OF *ORIENTIA TSUTSUGAMUSHI*

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Scrub typhus is an acute febrile illness caused by *Orientia tsutsugamushi* (*O. tsutsugamushi*) transmitted by bites of thrombiculid mites. The aim of this study was to investigate whether there are any differences in clinical features and severity between the Boryoung and Karp genotypes. Nested polymerase chain reactions (PCR) were performed with the blood buffy coats or eschars of patients with suspected scrub typhus who visited six hospitals from September to December 2006. We compared the clinical features and severity of illness in patients confirmed by nested PCR to have the Boryoung and Karp genotypes. Of 191 patients definitively diagnosed with scrub typhus, 168 were positive for nested PCR. Of these 168 patients, 133 were clustered as having the Boryoung genotype and 19 as having the Karp genotype. In this prospective study, the eschar detection rate was extremely high because of the thorough physical examinations carried out. Eschars and rashes were observed in 97% and 94% of the patients in the Boryoung group, but in only 73.7% and 68.4% of the patients in the Karp group, and the differences were statistically significant. However, there were no significant differences in complication rates, need for intensive care or mean lengths of hospital stay. In conclusion, our data indicate that the frequency of occurrence of eschars and rashes may depend on the genotype of *O. tsutsugamuchi*.

## 1022

### *MUTANS STREPTOCOCCI*: ANTIBIOTIC SUSCEPTIBILITY, CO-RESISTANCE AND SELECTION OF COTRIMOXAZOLE RESISTANCE AMONG PARTICIPANTS IN MULAGO DENTAL AND TASO CLINICS IN UGANDA

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The objective of this study was to evaluate the antibiotic susceptibilities of cariogenic mutans streptococci isolated from participants attending a dental and an HIV clinic in Kampala-Uganda, the antibiotic co-resistance and whether cotrimoxazole prophylaxis selects for resistance in these isolates. *In vitro* susceptibility to seven antibiotics were evaluated for 84 mutans streptococci (one isolate per person) from oral bacterial flora of 171 participants with dental caries. Isolates were confirmed by DNA analysis while the antimicrobial susceptibilities were assessed by E test and Kirby Bauer disc diffusion methods. Resistance to cotrimoxazole was also compared to 64 mutans streptococci (one isolate per person) isolated from 204 HIV positive participats taking cotrimoxazole prophylaxis. In the non prophylaxis group, 14.3% and 23.8 % of the isolates were resistant to cotrimoxazole and amoxicillin, respectively. Resistance to ceftriaxone, vancomycin, Chloramphenical, erythromycin and tetracycline was found in 46.4, 27.3, 14.3, 11.9 and 54.8% of the isolates, in that order. Isolates which were resistant to ceftriaxone were resistant to tetracycline (Wilcoxon Signed Ranks Test, Z=0.990, P=0.332) while strains which were resistant to erythromycin were also resistant to chloramphenical (Wilcoxon Signed Ranks Test, Z=-0.611, P= 0.541). The antibiotic resistance patterns of Streptococcus. mutans and Streptococcus sobrinus were similar (Kruskal-Wallis Test, P<0.005). Cotrimoxazole resistance was higher among the group on prophylaxis (54.7% Vs. 14.7%, Odds ratio: OR 7.24, 95%(3.10-17.21) (P is 0.000)) as compared to the non prophylaxis group. In conclusion, there was a high rate of resistance to Tetracycline and Ceftriaxone. The high frequency of non-susceptibility to tetracycline and Ceftriaxone among the mutans streptococci limits their use as therapeutic or prophylactic agents for diseases caused by these organisms. Also noted was that Cotrimoxazole prophylaxis selects for resistance in these isolates and thus a need for effective periodic surveillance of antibiotic susceptibility of the tooth decay causing streptococci.

## 1023

### A PILOT STUDY OF THE EPIDEMIOLOGY OF NEISSERIA MENINGITIDIS CARRIAGE IN CHILDREN IN BAMAKO, MALI

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Meningococcal meningitis outbreaks caused by *Neisseria meningitidis* are responsible for significant morbidity and mortality in the African meningitis belt. Asymptomatic pharyngeal colonization with *N. meningitidis*, known as carriage, is more common than invasive disease and the source of transmission. Yet, the epidemiology of carriage in this region is not

well understood. The African Meningococcal Carriage Consortium (MenAfriCar) aims to define the epidemiology of *N. meningitidis* carriage and evaluate a newly developed conjugate meningococcal serogroup A vaccine. Here, we describe the epidemiology of *N. meningitidis* carriage in children in Mali prior to the vaccine's introduction. We estimate the prevalence of carriage, assess potential risk factors, and evaluate two methods for determining carrier status. We conducted a cross-sectional pilot study of 250 children in Bamako, Mali. Eligible children were enrolled in school, 5-15 years old, healthy, and not vaccinated against meningitis for two years. Two oropharyngeal swabs were collected from each child; one by swabbing the posterior pharynx behind the uvula and the other by swabbing the posterior pharynx and one *tonsillar fossa*. Samples were processed using standard bacteriologic methods and 16S RNA sequence analysis to identify *N. meningitidis.* A questionnaire provided information about potential risk factors such as household size, smoking, and recent respiratory symptoms. The prevalence of *N. meningitidis* carriage among the 250 children (at least one swab positive based on 16S RNA analysis) was 21.2% (95% CI 16.3-26.8). The average age of carriers and non-carriers was similar (10.4 vs. 10.6 years). Carriers were more likely to be male (55% vs 49% of non-carriers), to live with < 8 people (36% vs. 27%), and to live in a house where no one smokes (51% vs. 36%). The swabbing methods had a high concordance (0.9) and a *kappa* of 0.61, denoting substantial agreement beyond chance. In conclusion, we found a high prevalence (21.2%) of *N. meningitidis* carriage among children in Bamako, Mali. Risk factors associated with carriage in Europe and the US did not appear to be associated with carriage in children in Mali, though further investigation is needed. Our results highlight the need for interventions that prevent or reduce meningococcal carriage, thus reducing transmission and preventing invasive disease.

## 1024

### DETECTION OF Q-FEVER SPECIFIC ANTIBODIES UTILIZING COM-1 ENZYME-LINKED IMMUNOSORBENT ASSAYS

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*Coxiella burnetii*, the causative agent of Q-fever, is a Gram-negative, obligate intracellular, and dimorphic bacteria. Acute Q-fever presents itself with flu-like symptoms, hepatitis, or pneumonia, and is usually a self-limiting disease with a low mortality rate. Chronic Q-fever, while less prevalent, often results in endocarditis, which has a much higher mortality rate. Since the symptoms of acute Q-fever are highly nonspecific, diagnosis can prove very difficult. The currently accepted method is indirect immunofluorescence assay (IFA) using the whole cell antigen. However, the process of isolating and purifying this whole cell antigen involves working with the highly dangerous *C. burnetii* in a Biosafety Level 3 lab (BSL-3), and the quality of the purified antigen is often inconsistent. Previously, six immunodominant antigens were identified by immunoblotting using two-dimensional gel separated whole cell antigens against patient sera. One of them, a 27 kDa outer membrane protein (Com-1) is *C. burnetii* specific. The recombinant Com-1 was purified and refolded to develop an enzyme-linked immunosorbent assay (ELISA). In this report the conditions for IgM and IgG detection using Com-1 in ELISA were optimized, and it was found that amplification using biotin tagged anti-human IgG and IgM along with steptavidin-HRP polymer could increase the signal and improve the sensitivity of the assay. Results from the optimized ELISA were very consistent with IFA data, indicating that the recombinant Com-1 could be used to replace the whole cell antigen for detection of Q-fever specific antibodies.

## 1025

### SEROSURVEY OF LEPTOSPIROSIS AMONG PATIENTS WITH ACUTE FEBRILE ILLNESS IN ACCRA

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The disease burden of some infectious diseases such as leptospirosis in Ghana has not been well defined because of difficulties in disease diagnosis and also because Malaria, which can have similar presentation and is hyper endemic in Ghana, may be mistakenly diagnosed instead. The Ghana Detachment of NAMRU-3 has recently completed a year-long study of acute febrile illness patients in Accra. 166 patients were enrolled in this study. Patients presenting at the hospital with fever lasting 2 days or more and a temperature of >38°C were examined. Those meeting enrollment criteria were informed of the study and signed an informed consent. Parents or guardians signed the consents for their children and assent was obtained from the children. Patients with obvious focal clinical diagnosis and children under the age of 4 were excluded. Patients, who met the AFI case definition, completed a case record form and blood samples were collected for malaria thick and thin film, blood cultures and serology. Positive results were confirmed at NAMRU-3, Cairo. The *Leptospira* IgM ELISA (PanBio Diagnostic's kit) was used as screening test for the diagnosis of acute leptospirosis. A value of 1.1 (according to manufacturer) was used as cut-off for further testing by MAT (Microscopic agglutination test). The MAT was performed on ELISA-positive sera to determine the most reactive *Leptospira* serogroups. A reactive MAT was determined by titer ranges, 1:200-1:25600. Of the 166 patients, 13 (7.8%) cases showed seroreactivity to *Leptospira* IgM by ELISA, 8 have so far been screened by MAT, 1 was reactive for serovar *Georgia* (1:3200) and 2 were reactive for the genus *L. Biflexa* but one showed reactivity for serovars *(Andamana*; 400) and *Bratislava* (400) and the other, serovars (*Andamana; 6400*); Bratislava (400) and Bataviae (400). The hospital diagnosis for 7 of these cases was malaria, even though the malaria smear results were all reported as negative. This suggests that almost 8% of patients that are diagnosed with malaria in our hospitals could have Leptospirosis. Leptospirosis in Ghana is currently underreported and more extensive study has to be conducted. This information is important to determine the disease burden of AFI etiologies, other than malaria, and to provide better treatment to patients in Ghana.

## 1026

### TEMPORAL TRENDS OF BURULI ULCER DIAGNOSES IN ANANEKROM, GHANA

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Buruli ulcer (BU), caused by the bacterium *Mycobacterium ulcerans*, is a devastating skin disease that can result in significant morbidity. It is endemic in tropical and sub-tropical regions in Western Africa including Ghana, where it is found in the southern part of the country. Within Ghana, the village of Ananekrom, located in Asante Province, is known to have the greatest number of reported cases (N = 34) during 2009. Using data collected by the Ghana Health Service, National Buruli Ulcer Control Programme during 2009, we examined the monthly temporal trends of diagnosed BU cases by clinical manifestation (nodule, plaque, edema, ulcer) and compare them with weather data (minimum average temperature, maximum average temperature, precipitation, normalized difference vegetation index). Clinical features were considered separately

and together given possible differences in the lag period between the development of clinical symptoms and diagnosis due to healthcare seeking behavior. From July to November, there was a gradual increase in the number of diagnosed cases. During the peak month of November (N = 8), half the cases were nodules and the other half were ulcers. Cross-correlation analyses allowed a comparison of lags between case counts and environmental variables through the year. A significant 2 month temporal lag was detected between plaque cases and ulcer cases. There was no correlation detected between any BU clinical manifestations and rainfall. A correlation between minimum average temperature and non-ulcerative cases was detected. Given the small numbers of cases, it is possible that elevated case counts may have been influenced by BU educational activities in the area. Understanding the temporal dynamics of the appearance of BU cases will help enable better identification of possible factors influencing patterns of infection, treatment, and reporting.

## 1027

### COMBINATION OF DNA VACCINE PLASMIDS CARRYING THE GENE CODING FOR THE TRUNCATED 47 KDA ANTIGEN AND THE CODON OPTIMIZED GENE OF 56 KDA ANTIGEN CAN PROVIDE EXCELLENT PROTECTION AGAINST THE HOMOLOGOUS CHALLENGE OF *ORIENTIA* KARP STRAIN IN A MOUSE MODEL

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Scrub typhus is an acute, febrile disease caused by infection with *Orientia tsutsugamushi*. At the present time there is no vaccine for scrub typhus. Western blot analysis of whole cell antigen with naturally infected patient sera revealed several potential antigens, including 56 kDa, 47 kDa and 110 kDa proteins. The 56 kDa protein appeared to be the most immunodominant protein which account for 10- 15% of the total amount of expressed proteins, making it one of the vaccine candidates. Recombinant protein r56Kp has been shown to provide excellent homologous protection of immunized mice. However, the protection provided by DNA plasmid VR1012 carrying the full ORF of 56Kp is poor. One of the possible reasons could be the low expression level of the 56 kDa antigen gene in mammalian system. In order to increase the expression level, the full length of 56 kDa antigen gene with optimized codon for mammalian expression was cloned into VR1012 (p56OptKp). Previously we have shown that the plasmid carrying the gene of 47 kDa antigen also provided very good homologous protection and some heterologous protection. However this 47 kDa antigen belongs to the family of HtrA and exhibits a very high sequence homology (46% identical, 70% semi-conserved, and 81% similar) with human protease HtrA1 in its central portion (aa 85-235). To avoid the concern of autoimmune responses for this vaccine candidate, we have successfully cloned a truncated fragment e (coding aa 236-477) into the VR1012 (p47eKp). The combination of p56OptKp and p47eKp at 1:1 ratio was evaluated for protective efficacy at two different doses (100 ug and 50 ug). Mice were immunized twice at four weeks interval and challenged at four weeks after the last immunization. The morbidity and mortality were monitored daily for 21 days post challenge. Close to 90% of the immunized mice were protected against the lethal challenge at different doses. These results strongly suggested that a successful vaccine formulation can be achieved by combining these two modified DNA plasmids.

## 1028

### A PREDOMINANT CLONAL COMPLEX OF VANCOMYCIN-RESISTANT *ENTEROCOCCUS FAECIUM* IS ASSOCIATED WITH THE LARGE VRE OUTBREAK IN RIO DE JANEIRO, BRAZIL

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Vancomycin-resistant *E. faecium* (VREF) strains have been worldwide reported among the leading causes of nosocomial infections. Since the emergence of VREF in Brazil, very few studies have been conducted to investigate phenotypic and molecular diversity of these strains in this country. We report the phenotypic and molecular characterization of VREF isolates from different Brazilian hospitals located in Rio de Janeiro state. VREF isolates, obtained from clinical sources or rectal screening in hospitalized patients seeking medical care at 15 hospitals over an 8-year period (2002 to 2009), were included in the study. Phenotypic characterization was based on conventional physiological tests. Antimicrobial susceptibility was determined by the disk diffusion method. MICs of vancomycin were determined by the E-test and the presence of *van* genes and *esp* gene was investigated by PCR. Genetic diversity was evaluated by pulsed-field gel electrophoresis (PFGE), using *Sma*I as the restriction enzyme, and by analysis of multiple-locus-variable number of tandem repeat (MLVA) for 6 genomic loci. *E. faecium* isolates harbored the *vanA* gene and expressed high-level resistance to both vancomycin and teicoplanin. All the isolates were resistant to ampicillin, erythromycin, imipenem, teicoplanin and showed high-level resistance to streptomycin and gentamicin. The majority of the isolates (>80%) was also resistant to ciprofloxacin and norfloxacin. All the isolates were susceptible to linezolid, nitrofurantoin and about 90% of the isolates were susceptible to fosfomycin. Resistance to tetracycline (about 6%) was due to the presence of the *tetO* and *tetM* genes. Both typing schemes were highly concordant and they identified a prevalent clonal complex (CC) designed as profile A by PFGE and MT12 by MLVA. The isolates belonging to the prevalent CC harboured the *esp* gene. These data strongly suggest the epidemic dissemination of a single CC of VREF in the area investigated.

## 1029

### CLINICAL MANIFESTATIONS OF SYPHILIS IN A RURAL UGANDAN HOSPITAL: ARE WE DOING ENOUGH TO DETECT SYPHILIS AMONG HIV PATIENTS IN AFRICA?

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Syphilis is still an important problem worldwide. With the advent of HIV/AIDS, syphilis became overshadowed and neglected resulting in its being missed in many patients yet the two diseases tend to coexist. Many people who are tested for HIV are not screened for syphilis, with the consequence of them suffering the complications of the disease undetected. For instance, 2 patients at our HIV clinic nearly lost their sight to previously undiagnosed syphilis manifesting as 'idiopathic chorioretinitis'. Objectives of this study were: 1) to re-awaken awareness of syphilis as a still important infection in Africa especially in HIV patients; and 2) to find if there is a difference in systemic symptoms and signs between HIV positive and HIV negative patients at our site. We randomly checked through case files of patients who had a final diagnosis of syphilis at discharge or death over the years 2002-2009.We looked at their age, gender, HIV status, syphilis status as well as symptoms and signs pertaining to the different organ systems-comparing the HIV positive with the HIV negative. Sixty-five subjects had both an HIV test and a confirmatory syphilis test. It is these 65 whose symptoms and signs that we compared. Of the 102 case files we reviewed, 85 had both a screening test result- RPR/VDRL plus the confirmatory TPHA. Eight had only a screening test- RPR/VDRL and 9 had

no evidence of a syphilis test in their charts. Seventy had an HIV test result, with 36 being HIV negative and 34 HIV positive while 32 had no HIV test result. Sixty-five subjects had both an HIV test and confirmed syphilis test with the summary as noted:Systemic symptoms/signs found:

Skin : 18.2% of HIV+s,18.8% of HIV-s Oral : 9% of HIV +s, 0% of HIV-s Musculoskeletal :18.18% of HIV+s,9.38% of HIV-s Respiratory:12.12% of HIV +s,18.75% of HIV-sCardiovascular :15.15% of HIV +s,15.63% of HIV-s.Neurological :51.5% of HIV+s, 31.25% of HIV-s Ocular : 0.0% of HIV+s,6.25% of HIV-s Abdominal :36.36% of HIV+s, 25% of HIV-s Genitourinary:3.03% of HIV+s,15.63 %of HIV-s,ENT :0% of HIV+s,and 0% of HIV-s. In conclusion: 1) syphilis is still a common and important disease, therefore routine testing for it is necessary by all clinicians; and 2) there were some differences in the frequency of different body systems affected in syphilis patients who are HIV positive versus those who are HIV negative. However with our data, the differences were not statistically significant. A bigger study is needed.

## 1030

### MOLECULAR EPIDEMIOLOGY OF GROUP A STREPTOCOCCUS AMONG CHILDREN AGED 5 TO 15

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Group A Streptococcus (GAS) is one of the most common and versatile human pathogens, causing superficial invasive infections as well as rheumatic fever and other immunological sequelae. Recently, multivalent M type-specific vaccines have shown promising results in trials. To use such vaccines in developing countries, the GAS burden and distribution of *emm*-types must be characterized. Four public elementary schools in two low -income quartiers (Djicoroni -Para and Sébénicoro) in Bamako Mali were identified and a census of the students was performed at the beginning of the study and at the beginning of the school year. Study personnel were present in each school infirmary to identify 5- to 15 year old children with pharyngitis and complete a clinical history and physical exam. A throat swab was obtained and processed to culture GAS according to standard procedures. *Emm*-typing was performed according to the Centers for Diseases Control and Prevention Protocol. All children with GAS pharyngitis were treated with a 10- day course of penicillin or erythromycin (if allergic to penicillin). From 30 May 2006 to 29 September 2009 of 12.500 students under surveillance per year 1757 presented with pharyngitis, 614 from Sébénikoro and 1143 from Djicoroni-Para. Of these 468 (26%) were positives for GAS. Almost half of the cases (61%) were over 10 years of age and most were female (60%). In addition to the classic symptoms which are pain (99. 8%) and difficulty swallowing (99.1%) others predominants symptoms such as fever (61. 5%), abdominal pain, (25. 4%), nasal running (38.5%), hypertrophied tonsil (74.6%), hypertrophy of anterior cervical lymph nodes (75.2%) were also present. Most of the children with GAS positive were sharing the bed with others children respectively 335 cases (71.5%) for 0 to 2 children in the same bed, 126 (27%) for 3 to 5 and 7 cases (1.5%) for those who were more than 6 children in the same bed. These data suggest that GAS is an important cause of pharyngitis in Malian schoolchildren. *Emm* -type distribution of pharyngitis cases appears to cover a broader age range. More data is needed to determine the burden of GAS infections and potentially introduce a vaccine.

## 1031

### LEPTOSPIROSIS-ASSOCIATED SEVERE PULMONARY HEMORRHAGE SYNDROME IS ASSOCIATED WITH ELEVATED IL-10 AND INCREASED CD19+ B CELLS AND ΓΔ T CELLS POPULATION

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Leptospirosis, a spirochete zoonotic disease, is increasingly recognized as an important cause of hemorrhagic fever. The objective of this study was to determine the cytokine profile and cell phenotype surface markers of patients with leptospirosis-associated severe pulmonary hemorrhage syndrome (SPHS), infected with *Leptospira interrogans*. Peripheral blood samples of leptospirosis patients with SPHS (n=5) and without (n=17) were examined for cytokine production using Cytometric bead array to measure the levels of IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IFN-γ and TNFα. Cell phenotype population of lymphocytes γΔ, CD3, CD4, CD8, CD19 surface markers were performed by FACSAria analysis. The major cell population of patients consisted of approximately γΔ T lymphocytes (5.8%), CD3+ (44.4%), CD4+ (21.43%), CD8+high (8%) and CD19+ (12.4%) cells. Additionally, patients with SPHS showed increased expression of CD86+ on CD19+ B cells. Production of anti-inflammatory cytokines, such as IL-10 and IL-4 was prominent in patients with SPHS. Interestingly, IFN-γ, IL-12 and TNF-α production was suppressed in the absence of SPHS. Furthermore, bacteremic load had no association with outcome response. In conclusion, our preliminaries results indicated that leptospirosis patients with SPHS presented Th2-like immune response while those without SPHS showed type-1 response.

## 1032

### ESTIMATE OF RISK FACTORS FOR LEPTOSPIROSIS IN CARTAGENA DE INDIAS - COLOMBIA

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Leptospirosis is a zoonosis of worldwide distribution, focused on the tropics, which can occur in both rural and urban centers whose transmission is integrated into the ecology of wild mammals and pets, as well as to the availability of water sources, neutral or alkaline soils and abundant rainfall in tropical countries. The aim of this study was to estimate the risk for transmission of leptospirosis based on the mortality in Cartagena de Indias - Colombia. The study was performed during the period November 2009 - March 2010, and applied on a sample of 20.003 dwellings, distributed in three locations of the city, by random sampling by cluster (MACO) multistage. It was designed a survey to identify risk factors for leptospirosis in the urban cycle, which included both socio-demographic variables, as the variables associated with risk of exposure and morbility. The main findings of this research are: The percentage distribution of employment shows that 60% of the population corresponds to students and housewives. Less than 3% of the inhabitants of the dwellings surveyed play occupations characterized as hazardous for leptospirosis. 40% of the population was in contact with animals. The analysis of risk factors based on morbidity, in terms of administrative zoning of the city, shows that living in the locality 3 is a risk factor in the transmission of leptospirosis, the town submitted an odd ratio of 3.4 (95% CI: 1,3 - 8,7). On the other hand living in the site 2 can be considered as a protective factor against disease (OR = 0.14, 95% CI:

0.019 to 1.0). The untreated water consumption appears to be a risk factor for leptospirosis (OR = 8.2, 95% CI: 1.1 - 61.6), the widespread practice of storing water for drinking is also associated with a failure to deal with it, even in locations where there is a continuous service. The risk associated to the environment showed two important factors: first, established a OR: 36.6 for contact with cattle with 95% CI 5.1 to 306.8. This finding is suggestive of the possible mode of transmission and risk of this infection in the city.

## 1033

### DIFFERENT OUTCOMES OF EXPERIMENTAL LEPTOSPIRAL INFECTION IN MOUSE STRAINS WITH DISTINCT GENOTYPES

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The mouse disease model has the advantage of a broad array of immunologic and genetic manipulation tools available for basic research. Some studies on transgenic and/or mutant mouse strains as models for experimental leptospirosis have been reported, however, the wider use of such models is hampered by our poor understanding of the outcome of experimental leptospiral infection among the different mouse strains available. We studied the outcome of infection by a virulent strain of Leptospira interrogans serogroup Icterohaemorrhagiae strain Cop in four commonly used wild-type mouse strains: A, CBA, BALB/c and C57BL/6. All infected animals received an intraperitoneal low inoculum (1.0 x 10e3) or high inoculum (1.0 x 10e6) in two independent experiments with 5-15 animals per group. Controls were inoculated with 1ml of sterile EMJH medium. The outcome endpoints evaluated in this study were survival, presence of kidney lesions, leptospire load in kidney samples, microscopic agglutination test (MAT) titre and anti-leptospiral IgG antibody levels. None of the mice strains were susceptible to lethal leptospirosis. However, several strains developed specific outcomes associated with sub-lethal leptospirosis. The difference of anti-Leptospira IgG levels between mice strains were significant in animals infected with lower inoculums with lower IgG levels observed in strain A. In both experiments and regardless of inoculum size, BALB/c mice produced lower levels of anti-Leptospira agglutinating antibodies, had a lower leptospiral load in kidney tissue and did not develop interstitial nephritis. Mouse strain A exhibited a high load of leptospires in kidney samples indicating that it may be the strain of choice for studies requiring large amount of leptospires recovered from murine renal tubules. Mouse strains CBA and C57BL/6 developed inflammatory lesions suggesting their use in studies on interstitial nephritis.

## 1034

### INTER-LABORATORY AGREEMENT OF PULSED-FIELD GEL ELECTROPHORESIS IDENTIFICATION OF *LEPTOSPIRA* SEROVARS

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Leptospirosis is a worldwide zoonosis caused by any of more than 250 *Leptospira* serovars. Serovar classification occurs through cross-absorption agglutination testing, a complex task which most laboratories cannot perform. The Centers for Disease Control and Prevention (CDC) developed a pulsed-field gel electrophoresis (PFGE) technique to identify *Leptospira* serovars. We measured its inter-laboratory reproducibility. A blinded exchange of 93 Leptospiraceae strains occurred between Brooke Army Medical Center (BAMC), who exported 36 strains, and Centers for Disease Control and Prevention, who exported 57 strains. Each strain was assigned a unique code at the providing institution. Exchanged strains included reference, clinical, and uncharacterized isolates. PFGE was performed with *NotI* using *Salmonella* Braenderup H9812 (digested with *XbaI*) as a standard. Gel images were analyzed with BioNumerics software and compared to patterns in each laboratory's database. The CDC database contained patterns of more than 800 strains; the BAMC database, more than 300. CDC identified 31 of 36 strains; 3 were misidentified (misID) and 2 did not match (noID) serovars in their database. BAMC identified 43 of 57 strains; 2 were misID and 12 were not in their database (noID). Overall, 93.7% (74 of 79) of strains present in each receiving laboratory's database were correctly identified. Exchange of gel images for noID isolates revealed most of them matched the pattern in the providing lab's database. Of the 5 misID isolates, 4 were not identified correctly as the reference strains were named differently, although patterns produced were the same. For the fifth isolate, the wrong serovar was sent to CDC. In conclusion, identification of leptospiral serovars by conventional methods is not readily available or practical. Molecular typing methods and equipment have become much more common in both research and clinical labs. The PFGE methodology showed good inter-laboratory reproducibility, but the integrity of reference databases is an issue with errors in *Leptospira* serovar identification.

## 1035

### COMMUNITY PREVALENCE RATE OF METHICILLIN RESISTANT *STAPHLOCOCCUS AUREUS* (MRSA) ASSOCIATED WITH PVL AMONG QATAR UNIVERSITY STUDENTS

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*Staphylococcus aureus* (MRSA).can cause infections ranging from mild to severe diseases that can be fatal. These infections occur between people works in hospitals, healthcare, and even the visitors of the hospitals. It is continuing to be the major nosocomial pathogen of Hospitals and community acquired. As a consequence, it was divided into two types either: Hospital Associated (HA-MRSA) or Community Associated MRSA (CA-MRSA) that may occur among healthy people in the wider community. Nasal swabs were obtained from female students at Qatar University. Swabs were inoculated into chromagel agar plates and incubated for 24 hours, pink coloured colonies were selected and appeared, for confirmation of identity *Staphylococcus aureus*. Final confirmation of MRSA, was done by susceptibility testing to cefoxitin through disk diffusion method. Out of the 514 samples, only 1 sample was positive for MRSA. The above isolate was sensitive to Ampicillin-Sulbactam, Cifazolin, Cefexime,Cefoxitin, Ceftriaxone, Oxacillin, Penicillin G, and Trimethomprim-Sulfamethoxazole. However its resistance was noticed to Ampicillin-Sulbactam, Cifazolin, Cefexime,Cefoxitin, Ceftriaxone, Oxacillin, Penicillin G, and Trimethomprim-Sulfamethoxazole. Now it is essential for hospital staff to follow the safety rules when they are dealing with patients, because the MRSA infections are transmitted to the community by contacting with hospital environment. This depends on maintaining their self hygiene because they can be the source of spreading MRSA among patients. The resistance of MRSA to antibiotics in Qatar is lower in comparing with Kuwait and Saudi Arabia. The sensitivity of MRSA to antibiotics is decreasing worldwide, and it is spreading between the people, becoming epidemic especially between school students, colleges, hospitals and any other places that people can gather. For this reason it is essential to do screening for schools in general, nurseries, civil workers and other universities in Doha and to do more genetic and molecular researches about MRSA in Qatar.

## 1036

### SPOTTED FEVER GROUP AND TYPHUS GROUP RICKETTSIOSES AMONG HOSPITALIZED FEBRILE PATIENTS IN NORTHERN TANZANIA, 2007-2008

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The importance of spotted fever group rickettsiosis (SFGR) and typhus group rickettsiosis (TGR) as causes of febrile illness in sub-Saharan Africa is unknown. In the first sub-Saharan Africa study of its type, we investigated the prevalence and correlates of SFGR and TGR in northern Tanzania. We identified febrile patients among consecutive admissions to two hospitals in Moshi, Tanzania, from September 2007 to August 2008, recorded standardized clinical data, and collected acute and convalescent sera. Acute SFGR and TGR were defined as a >=4-fold increase in IgG immunfluorescence assay titer to *R. conorii* or to *R. typhi*, respectively; a titer of >=1/64 defined SFGR or TGR exposure. Predictors and clinical management of SFGR and TGR were examined. Among 870 febrile patients, 449 (51.6%) had paired sera tested for acute SFGR and TGR; 828 (95.2%) had sera tested for SFGR and TGR exposure. Results suggested acute SFGR and TGR among 36 (8.0%) and 2 (0.5%) patients, respectively; 193 (23.3%) and 23 (2.8%) patients had results suggesting SFGR and TGR exposure, respectively. Among acute SFGR cases, the median (range) age was 15 (1, 77) years; clinical features included headache (66.7%), rigors (66.7%), and cough (61.1%). Acute SFGR was associated with leukopenia (OR 4.3, p=0.002) and serologic evidence of other zoonoses (OR 2.2, p=0.046). SFGR and TGR were never clinically diagnosed; the most common diagnoses among subsequently identified cases of acute SFGR were pneumonia in 14 (38.9%) and malaria in 12 (36.6%); 3 (8.3%) received antimicrobials active against SFGR. There was a protective effect of HIV against SFGR exposure (OR 0.36, p<0.001) and a trend toward the same for acute SFGR among those >=18 years (OR 0.32, p=0.071). No patients with SFGR or TGR died. SFGR but not TGR appears to be an important cause of febrile illness among inpatients in northern Tanzania; SFGR is likely endemic in this region. Clinical presentation of SFGR is nonspecific and appropriate antimicrobial treatment rare. The possible protective effect of HIV against SFGR warrants investigation.

## 1037

### DETECTION OF *LEPTOSPIRA* FROM SPIKED BLOOD AND URINE SAMPLES DRIED ONTO WHATMAN FTATM MATRIX CARDS

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Diagnosing leptospirosis is challenging and testing is often not available in remote settings where the disease is commonly contracted. Diagnostic testing is usually performed in reference laboratories, hindering the collection of samples early in the course of illness when testing may be more reliable. We evaluated the use of diagnostic PCR testing of serum and urine samples collected on Whatman FTA™ matrix cards (Piscataway, NJ) as a tool for remote diagnosis of acute clinical samples. If successful, this would allow for improved epidemiology data in addition to providing a means for delayed or remote diagnosis. 4 pathogenic and 1 saprophytic strains of *Leptospira* were grown in EMJH media at 30°C. Serial dilutions (1x107-1x102 organisms/mL) were prepared with 65uL of each dilution added to FTA™ cards. Identical dilutions of the 5 *Leptospira* strains spiked in human blood and urine samples were applied to FTA™ cards. 1 and 4 weeks after application, 2x2 mm squares were cut from each card, DNA extracted using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA), and conventional and real-time PCR testing performed using 16S and LipL32 primers. Pathogenic *Leptospira* strains were detected with both primers using both techniques. The lowest detection in pure culture was 1x104 with reliable detection at 1x106 and higher. In spiked blood and urine samples, positive results were seen 1x105 with consistent positivity at and above 1x106. Similar recovery rates were noted at 4 weeks. Good correlation was seen between pure culture and blood or urine samples. In conclusion, our results support the use of FTA™ cards as a convenient way to collect acute clinical samples from patients without access to care, providing maintenance of sample integrity for diagnostic PCR up to 4 weeks after collection. Our PCR technique required a large burden of organisms for detection, potentially limiting its use in patients without severe disease. If PCR sensitivity is improved, FTA™ cards could be a successful point-of-care collection technique for diagnosing leptospirosis.

## 1038

### EVASION STRATEGIES OF *ECHINOCOCCUS GRANULOSUS* TO TH1 HOST PROTECTIVE RESPONSE DURING HUMAN INFECTION

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Human cystic hydatid disease constitutes a major health problem in Algeria. More recently, we have highlighted an evident role of IFN-γ (Th1 cytokine) in parasite killing by NOS2 (Nitric oxide Synthase2) pathway. Moreover, IL-10 (Treg cytokine) production seems to be an evasive mechanism taken by the parasite to establish in the host by Arginase pathway, as reported previously. Of note, NOS2 and Arginase are known to compete for the common substrate, L-Arginine. Moreover, IL-10 downregulates IFN-γ production. Indeed, more researches are required to identify factors present in parasite cyst which affect protective Th1 response in *Echinococcus granulosus* human infection. We investigate the effect of laminated-layer (accelullar layer of hydatic cyst) extract (LLs) on Th1/Treg and NOS2/Arginase balance in culture performed with mononuclear cells (PBMC) of hydatid patients and healthy donors. Furthermore, we have investigated the effect of LLs on parasite viability in PBMC-parasite cocultures. Our results demonstrated that LLs reduced IFN-γ/NO production and enhanced IL-10 production and Arginase activity. In addition, LLs enhanced parasite survival *in vitro*. Similar findings are observed in cultures and cocultures performed with PBMC of patients and healthy donors. Moreover, the major antigenic fraction in LLs: the fraction 4 (12kDa, purified by chromatography) has the same effect as LLs. In conclusion, collectively, the present study provides evidence that *Echinococcus granulosus* laminated layer impairs Th1 protective response and allow the parasite to survive. Inhibition of these mechanisms seems to be important issue to address during the design of anti-hydatic treatment.

## 1039

### MUSCULAR HYDATIDOSIS IN TWO YOUNG MALES

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Hydatid cyst, caused by *Echinococcus granulosus* is a worldwide occurring infectious disease. Although diffuse internal localization of hydatid cysts is common, intramuscular localization has rarely been reported. In this text, we present two cases. In case 1, ultrasonography (US) of a 22 years

old male revealed a cystic mass in the paraspinal muscles. He didn't allow for surgical intervention. After the magnetic resonance imaging, PAIR (Puncture-Aspiration of cyst contents_Injection of hypertonic saline solution_Respiration) was used as a percutaneous treatment of hydatid cysts US guidance to the patient. Albendazole was given to patient before (7 days) and after (28 days) drainage. The cysts, examined after 7 days, a progressive shrinkage and solidification displayed. No allergic reactions or dissemination of cyst contents were discovered. In case 2, the hydatid cysts were localized in the lateral abdominal wall region. The patient was operated and treated with albendazole.In both cases, albendazole-induced elevations of liver enzymes were not determined.The cystic lesions with rare anatomic localizations require differential diagnosis, especially particularly in the endemic regions of hydatid disease. Following a positive diagnosis, a less invasive method can be applied. Percutaneous drainage treatment was efficient for both diagnosis and treatment in case 1.

## 1040

### THE WHO-CLASSIFICATION SYSTEM OF ALVEOLAR ECHINOCOCCOSIS OFFERS GUIDANCE FOR STRUCTURED TREATMENT

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Human alveolar echinococcosis (AE) is caused by the metacestode of Echinococcus multilocularis. The parasite forms multi-vesiculated lesions embedded in a dense fibrous tissue, infiltrates the primarily affected liver like a malignant tumor, and ultimately spreads into neighbouring or distant organs. The WHO-PNM-classification system encompasses the wide clinical spectrum by using 4 "P" categories for the distribution of lesions (P for parasite in the liver), 2 "N" and 2"M" categories for the presence or absence of local infiltration including lymph nodes (N) and metastasis (M), respectively. Stages I to IV are derived from those categories. The purpose of this study is to validate the classification system for surgically treated patients, and its usefulness to guide treatment for newly diagnosed patients. 144 patients (58 men and 86 women, mean age 52.7 and 48.7 years, respectively) were included. All of them were classified during the period from1998 to 2008 and had a median follow-up of 4.9 years (maximum 12 years). Treatment was provided according to the best knowledge. 59 patients underwent surgery, 85 were regarded as inoperable. All patients received benzimidazoles, and if necessary, interventional measures. Cure, stable disease, and progressive disease or relapse was assessed by imaging techniques including PET/CT scans, and by applying biochemical markers including serology. Of 144 patients 19 were grouped in stage I, 20 in II, 31 in IIIa, 36 in IIIb and 38 in IV. Thus, nearly half of the patients (stages IIIb and IV) had hepatic as well as extrahepatic disease requiring continuous treatment with benzimidazoles. Since surgery in combination with benzimidazoles offers cure, subgroups of patients in the different WHO stages were further analysed. Evaluable were 11 "surgical" cases in stage I, 14 in stage II, 12 in stage IIIa, 10 in stage IIIb, and 12 in stage IV, respectively. Kaplan-Meier plots clearly show a positive outcome for patients allocated in stages I (100%) and II (85%). In contrary, 70% of patients diagnosed in stages IIIb and IV relapsed or showed progression when taken off benzimidazoles after surgery. The subgroup of patients in stage IIIa followed an intermediate course. Thus, prospective classification into the WHO-PNM system clearly separates cases with favourable prognosis when radical surgery was applied, and thus, offers an appropriate basis for treatment decisions of alveolar echinococcosis.

## 1041

### *ECHINOCOCCUS MULTILOCULARIS* PHOSPHOGLUCOSE ISOMERASE (EMPGI): A GLYCOLYTIC ENZYME INVOLVED IN METACESTODE GROWTH AND PARASITE-HOST CELL INTERACTIONS

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In *Echinococcus multilocularis* metacestodes, the surface-associated and highly glycosylated laminated layer and molecules associated with this structure are believed to be involved in modulating the host-parasite interface. We report on the molecular and functional characterization of *E. multilocularis* phosphoglucose isomerase (EmPGI), which is a component of this laminated layer. The EmPGI amino acid sequence is virtually identical to its homologue in *E. granulosus*, and shares 64% identities and 86% similarities with human PGI. Mammalian PGI is a multifunctional protein that, besides its glycolytic function, can also act as a cytokine, growth factor and inducer of angiogenesis, and plays a major role in tumor growth, development and metastasis formation. EmPGI and multifunctional mammalian PGI share a typical motif that is absent in species without any extracellular function of PGI. Recombinant EmPGI (recEmPGI) is also functionally active as a glycolytic enzyme and was found to be present, besides the laminated layer, in vesicle fluid and in germinal layer cell extracts. EmPGI is released from metacestodes and induces a humoral immune response in experimentally infected mice, and vaccination of mice with recEmPGI renders these mice more resistant towards secondary challenge infection, indicating that EmPGI plays an important role in parasite development and/or in modulating the host-parasite relationship. We show that recEmPGI stimulates the growth of isolated E. multilocularis germinal layer cells *in vitro*, and selectively stimulates the proliferation of bovine adrenal cortex endothelial cells, but not of human fibroblasts and rat hepatocytes. Thus, besides its role in glycolysis, EmPGI could also act as a factor that stimulates parasite growth and potentially induces the formation of novel blood vessels around the developing metacestode *in vivo*.

## 1042

### CLINICAL CONTRIBUTIONS TO A NATURAL HISTORY OF ECHINOCOCCAL CYSTS

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The natural history of cystic echinococcosis (CE) of the liver is still incompletely understood.

Although the standardized ultrasound classification of CE introduced by the WHO Informal Working Group on Echinococcosis improved matters in this respect, the chronic nature of the disease makes it difficult to understand the exact sequence of changes unless short time intervals are used in follow-up. Clinical observations suggests that the sequence might be: CE1 --> CE3a --> CE4 in case of inactivation and CE1-->CE3a --> CE2 and CE4-->CE3b in case of chronicization (both CE2 and CE3b respond poorly to medical treatment or percutaneous drainage). We report two cases in which a CE3a cyst was seen "regressing" to a CE2 stage (transitional to active) and two patients in which a CE4 cyst changed into a CE3b cyst (inactive to transitional). This was possible thanks to continuous sonographic follow-up at short intervals, and both changes were seen shortly after albendazole treatment was discontinued.

The clinical implications of these findings will be discussed.

## 1043

### DIFFERENTIATION OF *DIPHYLLOBOTHRIUM LATUM* AND *D. PACIFICUM* BASED ON ITS2 SYBR GREEN REAL-TIME PCR

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Human diphyllobothriasis is a fishborne zoonosis associated with 14 different cestode species belonging to the genus, *Diphyllobothrium*. Recent data indicate that 20 million people are infected worldwide, despite the decline of cases observed in several countries, particularly in North America. This is probably due to the re-emerging pattern in different areas of the world including European, Asian and South American countries that did not have autochthonous diphyllobothriasis cases in years. *Diphyllobothrium lancelatum* and *D. latum* are the most prevalent species in North America while *D. latum* and *D. pacificum* occur in South America. Morphologic differentiation among *Diphyllobothrium* species requires examination of proglottids or scoleces, since eggs are morphologically identical. Nevertheless, differentiation of among species can be obtained on clinical samples with molecular methods. We developed and evaluated a SYBR Green real-time PCR assay using primers designed on the *Diphyllobothrium* ITS2 region. We evaluated this method on 15 clinical specimens containing *D. latum* (n= 8) and *D. pacificum* (n= 7) from North and South America, including four *D. latum* specimens from the 2004-2005 diphyllobothriasis outbreak that took place in Sao Paulo, Brazil, which is considered not to be endemic for diphyllobothriasis. Species differentiation was achieved with a dissociation curve analysis after the amplification. The differential melting temperature (Tm) of the *D. latum* using this method ranged from 81.5 oC to 82,0oC whereas for *D. pacificum* it ranged from 84.0 oC to 84.5 oC. By using this technique, results can be obtained 3 hours after the DNA is available with an average cost of $ 0.80 per sample. Ova-and-parasite (O&P) examinations can be reliably used in clinical diagnosis of diphyllobothriasis, but not for identification of the parasite at the species level, which is important in studies aimed at tracking the source of infections. For this purpose reliable and inexpensive molecular tools seems to be the best choice.

## 1044

### EVIDENCE OF ANTIBODY-MEDIATED IMMUNITY AGAINST NEUROCYSTICERCOSIS IN INDIANS

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Endemicity of an infectious disease associated with chronic exposure to the infective stage of the organism is linked to the development of acquired immunity that protects against infection. Taenia solium infections are endemic to India but the role of acquired immunity to the prevalence of disease has not been explored. In a study from south India, the prevalence of neurocysticercosis in the total population was found to be 0.13%. In a sample of the population who were free of seizures, taeniasis was detected in 0.8% of the population, Taenia cyst circulating antigens in 4.5%, IgG antibodies to Taenia ova antigens in 40.9% and IgG antibodies to infection specific T. solium cyst antigens in 15.9% of the population. In 93% of the seropositive population cysticercus antibodies were directed against low molecular weight cyst glycoproteins. These results show high exposure of the population to the parasite and a relatively high prevalence of active infections but a low prevalence of clinical neurocysticercosis. The findings may indicate that through constant exposure to low levels of infective Taenia ova the population acquires protective, antibody-mediated immunity to neurocysticercosis. The discussion will argue for a role of IgG antibodies to oncosphere proteins and low molecular weight cyst glycoproteins in protecting the population from neurocysticercosis.

## 1045

### THE MULTIPLE ANTIGEN BLOT ASSAY USING *TAENIA CRASSICEPS* PEPTIDES FROM 14 KDA GLYCOPROTEIN FOR NEUROCYSTICERCOSIS DIAGNOSIS

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Neurocysticercosis (NC) is caused by the presence of the larval form of *Taenia solium* on the central nervous system and represents the most severe form of the disease. The use of ELISA and EITB for detecting antibodies in cerebrospinal fluid and serum has been proposed for the laboratory diagnosis of NC. *Taenia crassiceps* represents an important experimental model and can be used for antigen preparation. The objective of this study was to standardize and evaluate the ELISA and LIA methods using synthetic peptides obtained from the *T. crassiceps* cysticerci GP14 glycoprotein to detect in patient's serum suspected of NC. It was prepared two biotinylated peptides from the GP14 sequence that was determined by our group using MALDI-TOF/TOF and MS/MS analysis. The results obtained from the ELISA and LIA-Pepbiot in patient's serum having NC were compared to the EITB and ELISA using antigens derived from *T. solium* and *T. crassiceps*. Three groups of patients were studied: NCA - 24 patients with NC confirmed by CT and MRI exams (7 active and 17 inactive NC) ; NG - 10 health individuals with no detectable parasitic disease and the OP - 43 patients with other parasites (OP). Considering the tests reactivity among the patients of group NCA it was observed that the EITB-Tso reacted with six of the 24 serum samples, the EITB-Tcra reacted with five, the ELISA-Tcra reacted with ten, the ELISA-Pepbiot with seven and LIA-Pepbiot with twenty. LIA-Pepbiot showed better results than the other tests. In the active NC six serum samples were positive, while five samples were ELISA-Pepbiot positive, only four samples were EITB-Tso and ELISA-Tcra positive, and three serum were EITB-Tcra positive. In addition, all patients with active NC were reactive at least with one test. The ELISA-Pepbiot and LIA-Pepbiot were negative in all serum from healthy individuals and patients with other parasitoses. The use of multiples synthetic peptides derived from *T. solium* and *T. crassiceps* could be an important tool for laboratorial diagnostic of NC.

## 1046

### *IN VITRO* MORPHOLOGICAL AND BIOCHEMICAL EFFECTS OF PRAZIQUANTEL AND ALBENDAZOLE ON *TAENIA SOLIUM* CYSTS

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Neurocysticercosis (NCC) resulting from *Taenia solium* (Ts) infections is a major cause of adult acquired seizures worldwide. Disease is caused by larval cysts, and treatment consists of anthelmintics drugs, albendazole (ABZ) or praziquantel (PZQ). There are no standard methods to assess drug activity to Ts cysts *in vitro*. Morphological, functional and biochemical changes that might reflect damaging (inhibiting, cytotoxic) drug effects

were analyzed after exposure of cysts to albendazole sulfoxide (ABZ-SO; the major active metabolite of the drug *in vivo*), PZQ, or combinations of both. PZQ exposure led to a decrease in cyst size and inhibition of evagination while ABZ exposure resulted in minimal changes. Alkaline phosphatase is normally secreted by cysts and both drugs inhibited AP secretion at concentrations of 5ng/ml and 50 ng/ml for PZQ and ABZ, respectively. Some combination of both drugs resulted in additive and/or synergistic activities. Parasite specific antigen, that can be detected in the CSF and blood of infected patients, is also normally secreted by Ts cysts *in vitro*. Antigen secretion was inhibited by ABZ and PZQ and a combination of both drugs in a manner similar to AP secretion, suggesting that inhibition of secretion is a common downstream consequence of the activities of both drugs. These studies establish quantitative methods to measure *in vitro* anthelmintic activity and suggest combination therapy with ABZ and PZQ may have clinical benefit.

## 1047

### SEROLOGICAL EVIDENCE OF ARBOVIRAL INFECTIONS AMONG HUMANS IN KENYA

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Outbreaks of arthropod-borne viral infections occur periodically across Kenya; however, limited surveillance takes place during interepidemic periods. Serosurveys triggered by outbreaks fail to detect potentially high levels of continuing exposure to these pathogens. Using sera from asymptomatic subjects collected across Kenya in 2000-2004, we assessed (via indirect immunofluorescence assay) prevalence of IgG antibodies to yellow fever (YFV), West Nile (WNV), tick-borne encephalitis (TBEV), dengue serotypes 1 - 4 (DENV1-4), and chikungunya (CHIKV) viruses. Seroprevalence estimates were YFV = 34%, WNV = 24%, TBEV = 14%, DENV1 = 51%, DENV2 = 53%, DENV3 = 44%, DENV4 = 36%, and CHIKV = 33%. Older individuals on the Indian Ocean were more likely to be seropositive than inland children. Among inland samples, lowland children were more likely to be seropositive for CHIKV (42% vs. 0%) than highland children. In Kenya, transmission of arboviral infection continues between known epidemics, remaining common across the country.

## 1048

### MOLECULAR EPIDEMIOLOGY OF THE SAINT LOUIS ENCEPHALITIS VIRUS IN THE BRAZILIAN AMAZON: GENETIC DIVERGENCE AND DISPERSAL

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Saint Louis encephalitis Virus (SLEV), a member of the genus Flavivirus (*Flaviviridae*,) is an encephalitogenic arbovirus broadly distributed in the Americas. Phylogenetic analysis based on the full-length E gene sequences obtained for 30 Brazilian SLEV strains was performed using different methods including Bayesian and relaxed molecular clock approaches. A new genetic lineage was described, hereafter named genotype VIII, which co-circulates with the previously described genotype V in the Brazilian Amazon region. Genotypes II and III were restricted to São Paulo state (Southeast Atlantic rainforest ecosystem). The analysis also suggested the emergence of the SLEV common ancestor between 91-189 years ago [Highest Posterior Density -HPD 95% 1875-1973], giving rise to two major genetic groups: genotype II, more prevalent in the North America, and a second group including the other genotypes (I, and III to VIII), broadly dispersed throughout the Americas, suggesting that SLEV initially emerged in South America and spread to North America. In conclusion, the current study demonstrated the high genetic variability of SLEV and its geographic dispersal in Brazil and in other New World countries.

## 1049

### ASTROCYTE ACTIVATION IN JAPANESE ENCEPHALITIS

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Japanese encephalitis (JE) is a major cause of childhood mortality and morbidity in Asia and the Western Pacific. Mortality rates can be as high as 30% with an additional one-third of survivors suffering severe permanent neuro-psychiatric disability. The neuropathic effects of JE virus (JEV) are still unclear; studies that address the mechanisms that produce inflammation and subsequent neuronal death are needed. While a number of studies have documented the importance of microglia in JEV infection, little is known regarding the role of astrocytes in brain inflammation. The objective of this study was to profile the role of astrocytes and their relationship to neuronal cell death in JEV infection. In a macaque model of JE, we used an immunohistochemical approach to characterize the role of astrocytes by testing for inflammatory markers including tumor necrosis factor (TNF)-alpha, interferon (IFN)-alpha, inducible nitric oxide (NO) synthase (iNOS), and matrix metalloproteinase (MMP)-2 and -9, as well as apoptosis pathways. In our study, we found that astrocytes undergo activation resulting in astrogliosis, produce TNF-alpha, IFN-alpha and MMP-2, and undergo apoptosis through the caspase-dependent intrinsic pathway. Our study confirmed for the first time *in vivo* that astrocytes play a crucial role in JEV infection by producing inflammatory mediators triggering bystander killing of neurons. More research is required to determine the precise role of astroglial activation and its implication on the neuropathogenesis of JE.

## 1050

### MODELING THE LONG-TERM NEUTRALIZING ANTIBODY PERSISTENCE IN ADULTS AFTER ONE DOSE OF LIVE ATTENUATED JAPANESE ENCEPHALITIS CHIMERIC VIRUS VACCINE

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Japanese encephalitis (JE) is the major cause of vaccine-preventable encephalitis in south-east Asia and the western Pacific. A live attenuated JE chimeric virus vaccine (sanofi pasteur, Lyon, France) has been shown to provide 87% seroprotection in 105 JE-naive adults 5 years post one-dose. Because long-term seroprotection data is essential for decision-making on need and timing of boosters, we applied linear and nonlinear statistical models to this data to predict neutralizing antibody titres (Abt) and seroprotection to 10 years post-vaccination. Data on subjects' Abt post one-dose were collected using plaque reduction neutralization test against homologous JE-CV at 0, 14, 28, 56 days, 6 months and then annually for 5 years and used to construct mixed effects statistical models. To avoid assumptions on the functional form of Abt decline, we constructed bi-exponential, linear, piecewise linear and exponential-type models from day 0 or day 28. The adequacy of model fit was based on statistical and heuristic criteria. Individual seroprotection was based on the accepted threshold of 1:10 /dilution units (Abt > 10). Observed Abt were found to rise rapidly by 28 days reaching geometric mean titres (GMT) of 247 (35.7-2136; 95% confidence interval) corresponding to 98.0% (93.1-100) seroprotection. GMT at 6 months declined rapidly to 128 (17.8-1313) corresponding to 95.0% (87.9-100) seroprotection before assuming a much slower rate of decline. The piecewise linear mixed model provided best fit amongst all models implying that long-term decline in Abt from 6 months remains linear. Predicted Abt at 10 years were 46.5 (23.8-91.2)

corresponding to 83.0% (71.6-94.4) seroprotection and average duration of protection of 29.7 years. Other model estimates for seroprotection at 10 years ranged between 66-93%. In conclusion, JE-CV seroprotection post 1 dose in adults is predicted to remain high for at least 10 years. A 5 year follow-up study is ongoing in children.

## 1051

### ASSOCIATION OF THE PROGRESSION OF LIVER FIBROSIS AND RESPONSE TO ANTIVIRAL THERAPY WITH FUNCTIONAL SINGLE NUCLEOTIDE POLYMORPHISMS (*TGF-B1, IFN-G, IL-6, IL-10* AND *TNF-A*) IN PATIENTS INFECTED WITH HEPATITIS C

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Cytokines play a key role in the regulation of immune responses. In HCV infection, the production of abnormal cytokine levels appears to contribute to the progression of disease, viral persistence, and affects response to therapy. Cytokine genes are polymorphic in specific sites, and certain polymorphisms have been shown to affect the overall expression and secretion of cytokines. The aim of the present study was to identify potential markers of cytokines genes associated with the progression of liver fibrosis and response to antiviral therapy.147 patients were enrolled (66 responders and 81 non-responders to antiviral therapy). 120 patients were stratified according to the stage of hepatic fibrosis (METAVIR index). Genotyping was carried out by PCR-SSP. The distributions of the following polymorphisms were compared in these groups: TNF-a(-308G/A [rs1800629]), TGF-b1 (codon 10 T/C [rs1982073], codon 25 G/C [rs1800471]), IL-10 (-1082 A/G [rs 1800896]; -819T/C [rs1800871]; -592A/C [rs 1800872]), IL-6 (-174G/C [rs1800795]), and IFN-g (+874T/A [rs2430561]). This study demonstrated a predominance of *IL-6* high producer phenotype in responders to antiviral treatment compared to the non-responders. No statistically significant difference was observed in allelic, genotypic and phenotypic frequencies of the *TNF*-a, *IFN*-g, *IL-10* and *TGF*-b*1* between these groups. When patients were stratified according to the METAVIR index, no statistically significant difference could be observed in these cytokine genes SNPs. These findings suggest an association between *IL-6* polymorphism in determining the therapeutic response. Further studies are required to determine the role of these polymorphisms on the progression of fibrosis in patients infected with HCV.

## 1052

### LACK OF CORRELATION BETWEEN SERUM AND SALIVA HEPATITIS C VIRAL LOADS

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Hepatitis C virus (HCV) can be detected in blood and other bodily fluids, such as saliva, semen and gastric juices. The latter are potentially alternate routes of transmission. Both qualitative and quantitative assays are important in the diagnosis of Hepatitis C and in monitoring a patient's response to therapy. The aim of the present study was to verify if there is correlation between HCV viral load in saliva and serum of infected patients. Mean viral RNA levels were 3.44 $\log_{10}$ in the saliva and 5.87 $\log_{10}$ in the serum samples. It was observed that saliva HCV viral load was significantly lower than serum. Also, there was no significant correlation between the HCV viral load this may indicate low transmission of HCV by saliva. However, this study demonstrates the importance of epidemiological studies to understand the significance of transmission of HCV and the need to evaluate use of saliva on the diagnostic and transmission of HCV.

## 1053

### SEROLOGICAL EVIDENCE OF CO-CIRCULATING DENGUE VIRUS SEROTYPES, AN UNDERREPORTED ARBOVIRAL DISEASE IN GUINEA

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Arboviruses have been studied in arthropods, bats, birds, and non-human primates in Guinea, but only rarely in humans, with the exception of Yellow Fever (YF), which has caused outbreaks roughly bi-annually since 2000. In order to determine the frequency of human dengue disease in the Guinean towns of N'Zerekore and Faranah, we utilized a plaque reduction neutralization test (PRNT) for testing human serum samples. The serum samples were taken from patients that presented to hospital with acute febrile illness, who were ruled not to have malaria or lassa infections. The samples were tested for dengue serotype-specific neutralization antibodies in order to ascertain the occurrence of acute or recent infections. In the case of early infections, the dengue PRNT test presents significant serotype cross-reactivity and therefore it is not always possible to identify the primary virus serotype after a secondary dengue infection. Dengue specific antibodies are present early in the infection and can generate lifelong immunity to the infecting serotype, but only a few months of cross protection to the other serotypes. We found that among the 151 individuals tested, 19% serum samples had greater than 80% neutralization by one specific serotype, in which 33.3% sera samples reacted positively with dengue 1 virus, 33.3% samples reacted with dengue 2, 10% samples reacted positively with dengue 3, and 23.3% of samples with dengue 4. Furthermore, 21% samples had no detectable neutralization for any of the dengue serotypes, 3% bound with greater than 80% neutralization on all serotypes and the remaining 57% of samples cross reacted in diverse combinations of dengue virus 1, 2, 3, and 4. Endpoint titrations and cross neutralizations against YF, Zika, Usutu, and Koutango viruses are underway for these serum samples. These results strongly suggest that dengue, at least, and perhaps other various arboviruses, are circulating in and likely the cause of human disease in Guinea.

## 1054

### THE THREAT OF WEST NILE VIRUS TO THE GALAPAGOS ISLANDS, ECUADOR

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West Nile Virus (WNV) has impacted the health of humans, domestic animals and wildlife across North America since it arrived to the Americas in 1999 to New York City(1-3). Knowledge of the distribution and impact of WNV in South America has been limited to serological assays from Venezuela and Columbia(4, 5), with virus isolation achieved in Argentina in 2006(6). An imminent threat exists to the Ecuadorian islands of Galapagos located 600 miles west of continental South America. I am investigating this risk as part of my PhD of which I am now in the 3rd final year. WNV is maintained in an enzootic 'mosquito vector - avian host' cycle. My studies examine the serological status of Galapagos birds, and the vector ecology of further 2 species of mosquitoes - *Aedes taeniorhynchus* and *Culex quinquefasciatus*, in pertinence to the threat of WNV impacting Galápagos. *A. taeniorhynchus* of Galápagos established across the archipelago before human habitation and are genetically distinct from other strains of this species found elsewhere in the world(7).

C. *quinquefasciatus* alternatively is a relatively recent introduction and a disease vector concerns(8). Abundance and distribution of both mosquito species in Galápagos and their ecological characteristics including feeding preferences are being researched. Historical isolation of Galapagos has produced a range of endemic birds, mammals and reptiles. This exceptional island fauna is likely 'immunologically naïve' having been sheltered from disease exposures prior to introduction of invasive species including pathogens(9). Galapagos is economically important for Ecuador due to the growing number of overseas and national visitors attracted to the archipelago for nature tourism. The islands themselves have developed and now have over 30,000 human inhabitants - populations that have been impacted by another flavivirus, Dengue, with the arrival of a third mosquitoes species to Galapagos, anthrophillic *Aedes aegypti*. In 2009 and during 2010 I examined the capacity of both *A. taeniorhynchus* and C. *quinquefasciatus* to become infected with, and transmit WNV. This Vector Competency work takes place in the USA using field mosquitoes collected in Galápagos. Seasonality in Galápagos is represented by conducting experiments at two temperatures. The capacity of Galapagos mosquitoes to transmit WNV and subsequent consequences for this tropical region will be discussed.

## 1055

### CULEX FLAVIVIRUS ENHANCES WEST NILE VIRUS MOSQUITO INFECTION, CHICAGO USA

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*Culex* flavivirus (CxFV) is an "insect-specific" Flavivirus globally distributed in mosquitoes of the genus Culex. CxFV was positively associated with West Nile virus (WNV) infection in a case-control study of 268 mosquito pools from a focus of WNV transmission in Chicago, USA. CxFV infection rates were high (approximately 100 infected mosquitoes per 1,000 tested), and WNV-positive pools were 4 times more likely to be infected with CxFV than spatiotemporally matched WNV-negative pools. Among WNV-positive individual mosquitoes, 6/15 (40%) were also CxFV-positive, demonstrating that these two flaviviruses co-infect mosquitoes in nature. These results challenge the hypothesis of "super-infection exclusion" in Flavivirus infection and, by contrast, demonstrate an unexpected positive association between CxFV and WNV. Additional analyses suggest that CxFV may be heterogeneously distributed across urban land cover types within the study area, perhaps reflecting variation in mosquito community dynamics. This study provides evidence that CxFV may enhance WNV infection in mosquitoes that are epidemic "bridge vectors" of WNV to humans. Insect-specific flaviviruses such as CxFV may therefore indirectly influence human disease risk.

## 1056

### STABILITY OF REAGENTS ASSOCIATED WITH MICROSPHERE IMMUNOASSAYS OVER TIME USING DIFFERENT STORAGE SOLUTIONS

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The DVBD/CDC in Fort Collins, CO, previously developed microsphere immunoassays to detect IgM to West Nile virus (WNV), St. Louis encephalitis virus (SLEV) and eastern equine encephalitis virus (EEEV). Related assays are being designed with some changes in methodology including the buffering system. As these assays are shared with State health departments, questions regarding the stability and storage requirements for the associated reagents arise. Currently, stock solutions of antigen/antibody-coupled microspheres, and control sera, are prepared and stored at 4ºC for up to 1 month. Working dilutions are prepared immediately prior to use. This time limit was derived from experiments performed during assay development. Storage times greater than this, or alternate storage buffers, were not previously investigated. The convenience factor would be significant if stock or working dilutions could be made in larger volumes to be used over longer periods without deterioration. In addition, less frequent stock preparation could reduce run-to-run error. Using the new methodology, 4 separate series of experiments were performed over the course of 9 months, using buffers manufactured by Candor Bioscience Gmbh, designed specifically to improve stability and test performances. These 4 experiments included: 1) WNV and EEEV antigens were used to illustrate the stabilities of recombinant and suckling mouse brain antigen preparations, respectively, in 4 buffer systems. 2) Positive serum controls to WNV and EEEV were used to illustrate the stabilities of anti-flavivirus and anti-alphavirus sera, respectively, in 3 buffer systems. 3) The effects of lyophilization of the microspheres after reacting antigens with antibody-coupled bead sets were investigated. and 4) The use of an alternate blocking solution in the coupling method was investigated. The results of these experiments are presented, and will be used to finalize methodology for a new generation of arboviral serologic tests. In addition the information may prove useful in knowing how best to provide for long-term storage and shipment of these reagents.

## 1057

### WEST NILE VIRUS RISK ASSESSMENT FOR FOUR MIDWESTERN URBAN AREAS

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Comprehensive knowledge of mosquito populations in urban areas is critical to determining which species pose the greatest risk of West Nile virus transmission to humans. Because mosquito species vary considerably in behavior and ecology, this information is key to directing surveillance and abatement resources for maximum efficacy. Using a previously established WNv risk model and measures of relative mosquito abundance, we determined that Aedes vexans may pose the greatest risk of WNv transmission in the Madison, Wi area. This species accounts for more than 80% of the risk while *Culex* pipiens, usually thought of as the major vector for human WNv transmission in the Midwest, accounts for less than 10% of the risk. Application of the model with local mosquito abundance data for Minneapolis Minnesota also implicates A. vexans as a potential key vector for this area, while the highest risks for Des Plaines, Illinois and for Milwaukee, Wisconsin, come from *Culex* pipiens. The model accurately predicted human WNv infection rates for three of the four urban areas in the study.

## 1058

### TRANSMISSION, AMPLIFICATION, AND EVOLUTION OF WEST NILE VIRUS IN CHICAGO, USA

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The west suburbs of Chicago, USA, have been a persistent focus of West Nile virus (WNV) transmission since 2002. In this area, WNV undergoes predictable seasonal amplification, characterized by peaks of mosquito infection and human cases in late summer. This multi-year study examines how fine-scale ecological processes within the urban landscape mediate patterns of viral transmission and amplification, and how these outcomes

influence viral evolution locally. Birds and mosquitoes were trapped within an approximately 100 km2 study are each year between 2005 and 2009 and tested for WNV using serological and molecular methods. Viral genome sequence data were used to examine patterns of viral evolution within the study area. Results indicate that WNV amplification in suburban Chicago is disproportionately influenced by a few relatively common bird species, such as the American robin. The mosquito *Culex pipiens* functions as both epizootic and epidemic vector, owing to its selection of both birds and mammals as blood hosts, which in turn reflects the species' complex populations structure. Preliminary field data on avian distributions, coupled with spatially explicit models of WNV transmission, indicate the potential importance of aggregated host distributions (especially nighttime roosts) to localized WNV transmission and persistence within the study area. Microclimate, features of the built environment, and co-circulating pathogens also appear to influence WNV transmission, perhaps explaining the heterogeneous distribution of WNV within the study area. Finally, molecular phylogenetic and phylodynamic analyses provide evidence of distance-limited viral transmission, viral genetic diversification, and fine-scale variation in viral genetic variability across suburban land cover types. Together, these lines of evidence suggest that WNV transmission is spatiotemporally heterogeneous within suburban Chicago. "Hot spots" of arboviral transmission in urban settings may actually represent the coarse-scale aggregation of highly localized ecological interactions.

## 1059

### ALTERED EXPRESSION OF PRO-INFLAMMATORY CYTOKINES: MECHANISMS UNDERLYING MINOCYCLINE-MEDIATED PROTECTION AGAINST WEST NILE VIRUS (WNV)-ASSOCIATED ENCEPHALITIS (WNVE) IN MICE

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West Nile Virus, WNV, an emerging viral pathogen in the United States causes potentially fatal encephalitis. However, no therapeutic drugs or vaccines are available to prevent or treat WNV infection or its neurological sequelae in human. A recent study demonstrated that minocycline, a broad-spectrum antibiotic, protected mice from Japanese Encephalitis Virus (JEV)-associated encephalitis. However, the role of minocycline in WNVE is unclear. Age and gender matched, 8- to 12-weeks old C57BL/6 mice were inoculated with 1,000 plaque-forming unit (PFU) of WNV (lineage I, NY99), and were either untreated or treated with minocycline and monitored for viremia and survival for up to 3 weeks after infection. Viremia was quantitated by plaque assay, mice survival was analyzed using Log-rank (Mantel-Cox) tests and serum cytokines were quantitated by Luminex assay. Almost 90% mice succumbed to death by day 10 after WNV infection. Viremia peaked on day 3 after infection and clinical symptoms including hind-limb paralysis and hunchback were observed starting at day 7. Interestingly, 30% of WNV-infected mice treated intraperitoneally with minocycline, 40 mg/kg/day, starting on day 3, survived. The survival curve was significantly different between WNV-infected -untreated and -treated mice (Chi-square 6.142, df 1, $p < 0.0132$). There was no difference in the viremia between the mice untreated or treated with minocycline and viremia had no direct correlation with CNS viral load or mortality. Minocycline up-regulated the expression of IL-1β, IL-6, TNF-α and MCP-1, down-regulated IL-12p70 expression, whereas the expression of IL-10 was not altered. In conclusion, minocycline promoted significant survival of mice without altering the viremia. While the precise mechanism of minocycline-associated protection is unclear, minocycline alters the expression of several pro-inflammatory cytokines and it is the intricate balance in the expression of these cytokines that determines ultimate outcome from WNV-associated encephalitis.

## 1060

### WEST NILE VIRUS BINDS TO RED BLOOD CELLS OF SEVERAL VERTEBRATE SPECIES

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West Nile virus (WNV), a mosquito-borne flavivirus, is the most common cause of viral encephalitis among humans in the US. WNV can be transmitted by blood transfusion, and we have reported that WNV binds to red blood cells (RBC) and remains infectious to Vero cells. Since WNV infects a broad range of vertebrates, we have investigated the ability of WNV to bind to RBC from various vertebrate specimens using classical hemagglutination assays (HA) and detection of bound virus by TaqMan RT-PCR amplification. RBC from 18 different vertebrates including human, mouse (BALB/c and C57Bl/6), rat (7 species), rabbit, sheep, ox, calf, sheep, pig, horse, and chicken. HA was performed on a series of WNV dilutions over a pH range of 5.75 to 7.2. To quantify WNV binding at pH 6.2 and 7.2 we performed TaqMan on human, ox, calf, and sheep RBC. For most samples, the optimal pH for HA varied between 6.2 and 6.4. Mouse RBC, from both strains, agglutinated most strongly at pH 5.75. HA in Sprague-Dawley rats was noticeably weaker than HA in cotton rats, occurring over a narrower range of pH and WNV concentrations with no wells showing complete agglutination. Calf RBC agglutinated weakly, and no HA was observed with ox RBC at any pH or viral concentration. Taqman assays performed on calf and ox RBC detected WNV associated with RBC at viral concentrations for which no HA was observed.

The degree to which RBC can be agglutinated by WNV varies between the different animals tested. In addition, WNV HA is dependent on pH, and the optimal pH range of HA also varies by species. RBC from bovine species show significant variation in surface sialic acid content, and the poor performance of bovine RBC in HA and binding assays is consistent with our earlier observations regarding inhibition of WNV-induced HA of human RBCs by sialic acid. Continued comparisons of RBC biochemistry and surface antigens, especially between RBC from oxen, cotton rats, and Sprague-Dawley rats, may yield insights into the nature of the molecular species that mediate WNV-RBC attachment.

## 1061

### EXPERIMENTAL EVOLUTION OF WEST NILE VIRUS EVALUATED *IN VIVO*

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Arboviruses perpetuate through alternating replication in both vertebrate and invertebrate hosts. The trade-off hypothesis suggests that these viruses maintain adequate levels of replication in two hosts in exchange for superior replication in one host. Releasing the virus from the constraints of a two-host cycle should thus facilitate adaptation to a single host. This theory has been addressed in a variety of systems, but remains poorly understood. We therefore sought to determine the fitness implications of alternating host replication by serially passing WNV twenty times (a) exclusively in mosquitoes (b) exclusively in chicks or (c) back and forth between mosquitoes and chicks. Passed viruses were then competed *in vivo* in fitness assays against a marked reference virus in both mosquitoes and chicks. The ratio of experimental WNV to reference WNV in total virus output was detected by RT-PCR followed by quantitative sequencing and the 'winner' was deemed to have a replicative fitness advantage. Exclusive serial passage in mosquitoes resulted in improved replication in mosquitoes and decreased replication in chicks compared to the co-infected reference virus. Control competitions using viruses passed alternately between chicks and mosquitoes showed no significant difference in ratio of experimental WNV to reference WNV in total virus

output from either host compared to inocula. Consensus sequences for single-host specialized WNV will be examined for sequence elements that can be correlated to either improved or reduced replicative fitness. Additionally, we are conducting fitness restoration studies to evaluate whether fitness in the bypassed host can be regained through alternate passage regimens. Concurrent studies in our lab competed populations of WNV having various levels of genetic diversity against the reference WNV in mosquitoes and chicks. These studies demonstrated that higher levels of genetic diversity were associated with increased replication fitness in mosquitoes but not chickens. Collectively, these results emphasize the importance of virus replication in mosquitoes to WNV adaptation and evolution in North America.

## 1062

### THE IMPACT OF MORTALITY IN BIRDS ON INCIDENCE OF WEST NILE VIRUS HUMAN NEUROINVASIVE DISEASE

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West Nile virus (WNV) is associated with high mortality in some North American birds, such as corvids. Survival of WNV infection usually leads to sterilizing immunity. The threshold theorem (Kermack&McKendrick, 1927) states that transmission is only supported if the density of immune hosts in the population does not exceed a critical level. WNV-associated avian mortality might thus counteract the dampening effect of increasing avian population immunity on WNV transmission. I present results of an analysis of the relationship between WNV Human neuroinvasive disease (WNNID) annual incidence in ten US states (CA, CO, FL, IL, LA, MA, MD, MN, SC, TN) and indicators of WNV transmission and bird mortality in the previous year. This analysis is based on data from the North American Breeding Bird Survey (annual bird abundance) and annual WNNID incidence data, reported by the CDC. Results from this analysis will contribute to improved prediction of WNNID incidence.

## 1063

### INTEGRATED CONTROL OF MALARIA AND HELMINTHS THROUGH UGANDA'S HEALTH SYSTEM

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Intestinal worms and schistosomiasis cannot be eliminated unless there is universal access to safe water and sanitation, however, such improvements are likely to take several decades in many African countries. In the interim, the mainstay of control is regular anthelmintic treatment. In recent years the Ugandan Ministry of Health has implemented mass treatment through community and school-based programmes. Concomitantly, donors have increased their support to malaria control including strengthening health systems to ensure that rapid diagnosis, effective treatment and appropriate prevention for malaria are routinely delivered at the facility level. Here there is an obvious opportunity to build on these investments to also integrate worm control into the existing health system, but evidence in practice is limited. To sustain the impact of past and present investment into deworming campaigns in Uganda, delivery of anthelmintics needs to become an integral part of routine health care delivery. A pilot intervention to support health facility based malaria, STH and schistosomiasis control is being implemented in Bulisa and Kibaale districts in western Uganda. We report results of an evaluation of this programme. Results from a baseline needs assessment of health facilities show that very few health workers had ever received training or supervision on STH or schistosomiasis case management. Most had no access to guidelines for diagnosis or treatment, resulting in inconsistency of deworming strategies between facilities. Pregnant women rarely received deworming treatment due to concerns about safety of anthelmintics in pregnancy. There was minimal interaction between mass treatment campaigns and facility-based health workers, with many facility staff believing responsibility for deworming lies with campaigns only. Subsequently, a package of interventions to improve health worker performance in 40 health facilities is described, including job aids, training and initiation of an external supervisory process. Results of the follow-up assessments are also described.

## 1064

### A SURVEY OF SOIL-TRANSMITTED HELMINTHS AND LYMPHATIC FILARIASIS IN SIX PROVINCES OF PAPUA NEW GUINEA

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During the setting up of sentinel sites for future monitoring of a national lymphatic filariasis elimination and school-age deworming program, 300- 500 blood and faecal samples are being collected at two sites in each province of Papua New Guinea. This poster provides the results obtained in Bougainville, East and West New Britain, Gulf, New Ireland, and Oro provinces. Faecal samples were examined by microscopy for soil-transmitted helminths, and night blood samples were tested for filarial antigen by the ICT test (and in some instances by Og4C3 ELISA) and microfilaria on a 60ul slide. Hookworm was found at all 1 sites with the prevalence varying from 86-21%. Ascaris lumbricoides was found at 6 sites with the prevalence varying from 39 to 0.3%. Trichuris trichiura was also found at 6 sites with a prevalence between 24 and 0.3%. Filaria antigen positives were found at all sites with a prevalence between 57 and 6%, all but two sites also contained microfilaraemics with a prevalence of between 24 and 11%. These results confirm historical data that Papua New Guinea has a high prevalence of soil-transmitted helminths and lymphatic filariasis and requires a nation-wide integrated campaign to combat these parasites.

## 1065

### THE TRIPLE CO-ADMINISTRATION OF ALBENDAZOLE, IVERMECTIN AND AZITHROMYCIN IS SAFE IN A LYMPHATIC FILARIASIS AND TRACHOMA CO-ENDEMIC AREA OF SIKASSO, MALI

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Neglected tropical diseases (NTDs) are coendemic in many areas of the world, including subsaharan Africa. Consequently, financial and logistical benefit can be gained from integration of preventive chemotherapy programs (PCPs) in such areas. To assess the safety of this approach for the co administration of azithromycin, albendazole and ivermectin, 4 villages in two lymphatic filariasis (LF) and trachoma coendemic districts of Sikasso, Mali, were randomly assigned to triple therapy or standard therapy (albendazole plus ivermectin (A/I) followed 1 week later by azithromycin). These villages had previously undergone 4 consecutive yearly mass drug administration campaigns with A/I and 2 with azithromycin. One village was randomly assigned to each treatment arm in each district. After a baseline assessment, study drugs were administered under direct observation in the clinic. Subjects were encouraged to return to the clinic at any time during the 14 day treatment period to report adverse events and were seen in the clinic on days 7 and 14.The total population of the two villages was 9109, of which 7515 were eligible for treatment (age >5 years). A total of 3016 subjects participated in the study (40.1% of

the eligible population). No serious adverse events occurred during this pilot community trial, and all observed AEs were mild in intensity (mainly diarrhea, headache, abdominal pain, nausea, vomiting). The number of subjects that reported at least one AE was significantly higher in the triple co-administration group (15.75%; 238/1511) as compared to the standard treatment group (18.98%; 286/1507) (OR= 1.26; 95% CI (1.04-1.53); p=0.018). Of note, the overall frequency of AEs in the triple therapy group, 18.98% (286/1507), was comparable or lower than published frequencies of AEs for A/I alone. These data suggest that coadministration of A/I and azithromycin is safe. Additional analyses, including stratification by gender and age group, are currently underway.

## 1066

### ANEMIA, IMPAIRED GROWTH AND EXERCISE INTOLERANCE IN KENYAN CHILDREN: THE ROLE OF SCHISTOSOMIASIS AND POLYPARASITISM

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To quantify burden of disease among children living in areas endemic for multiple parasites, we conducted surveys of affected villages in coastal Kenya. The objectives of the study are to measure the co-prevalence of *S. haematobium*, filariasis, malaria, hookworm, and other geohelminths among residents 5-18 yrs old, and to determine the relationship between parasite load and co-infections with the morbidity outcomes of anemia, reduced fitness, and undernutrition. Cross-sectional data were obtained from three villages during the months of April, August, and November 2009, respectively. After diagnosis (based on urine, stool and blood testing) participating children underwent standardized anthropometric measurement and performed a 20 m shuttle-run fitness test. Results to date from 1382 children reveal some significant heterogeneity among villages. Schistosomiasis prevalence was high in Nganja and Milalani (62 %) and lower in Vuga (25 %). Malaria (by ICT) was variable according to season, ranging from 8%-18%.Filariasis was most prevalent in Vuga (16 %). However, anemia was highly prevalent (50-54%) in all villages, and had a strong association with heavy-intensity schistosomiasis in both high and low prevalence villages. Low-intensity schistosomiasis was also a significant correlate of anemia in Vuga, suggesting a role for infection-related 'anemia of inflammation'.Synergy between infections was observed for hookworm-malaria in anemia, schisto-filaria and schisto-malaria for stunting in Milalani and schisto-filaria and hookworm-*Trichuris* for severe malnutrition in Nganja. Fitness correlated with hemoglobin level and older males were more stunted and wasted in 2/3 villages. We conclude that regardless of location-specific differences between villages (season, slope and nutrition), polyparasitism represents a collective threat to children's health and integrated control approaches appear warranted. Ongoing studies, involving antibody testing for past exposures,malaria PCR and cytokine testing for parasite-mediated inflammation, will refine prevalence estimates and immune response pathway associations.

## 1067

### EVIDENCE TO SUPPORT THE INCLUSION OF VECTOR CONTROL STRATEGIES IN THE GLOBAL EFFORT TO ELIMINATE LYMPHATIC FILARIASIS

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The Global Programme to Eliminate Lymphatic Filariasis (GPELF) advocates for 4-6 rounds of annual mass drug administration (MDA) to interrupt transmission. Although vector control is not currently a part of the strategy, implementation of vector management plans stands to greatly reduce lymphatic filariasis (LF) transmission and could shorten the time frame for elimination in a given area. Here, we present evidence that the use of long-lasting insecticide treated nets (LLINs), distributed as part of the Global Fund to Fight AIDS, Tuberculosis, and Malaria (GFATM), greatly reduced transmission of lymphatic filariasis in five villages in the East Sepik Province of Papua New Guinea (PNG). Anopheline mosquitoes were collected by human landing catch for four nights/month for one year prior to and one year following LLIN distribution (n=6,898). Half of the samples were dissected and larvae of *Wuchereria bancrofti* were identified under the microscope. The other samples were used for DNA extraction and identification of *W. bancrofti* by PCR. A survey conducted shortly after bednet distribution indicated that 83% of people slept under a bednet the previous night (n=2,459). The man biting rate was significantly reduced ($p<0.001$) post LLIN distribution. In addition, the proportion of mosquitoes identified as infected with *W. bancrofti* by PCR dropped from 15.3% to 4.9% (p=0.02). While 0.6% of mosquitoes were identified as infective pre-LLIN (n=3,935), zero mosquitoes have been found to be infective post-LLIN (n=236). In PNG, where anopheline mosquitoes are the primary vectors, LLINs have proven quite effective in preventing transmission of *W. bancrofti* from human to vector. With the difficulties faced by many LF endemic countries in supporting an MDA program for an extended period of time, integrated vector management may improve durability of programs to eliminate the disease.

## 1068

### EVALUATING PRIMARY HEALTH CARE DELIVERY SYSTEMS FOR NTDS IN WESTERN KENYA

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Neglected tropical diseases affect over one billion people in the tropics. Even though effective drugs exist for most of these diseases, they do not effectively reach majority of the persons who need them. Part of the challenge is how to get such existing interventions to affected communities. There is, therefore, an urgent need for better evaluation of the effectiveness of different delivery strategies in achieving and sustaining high population coverage at adequate quality levels. The ongoing study intends to test if the Community Directed Intervention (CDI) approach, that has almost eradicated onchocerciasis in endemic countries in Africa, could be used to strengthen the Primary Health Care (PHC) system in rural Kenya. In the first phase of the study, we have analyzed the prevailing PHC practices in western Kenya, including the Community Strategy (CS) and its ongoing implementation. Study districts in western Kenya were chosen on the basis of PHC delivery and general development levels. These included Rarieda, Kisumu West, Rachuonyo and Homa Bay Districts. Kisumu West and Bondo districts are in the relatively more developed Central Nyanza where PHC delivery is considered average for Kenya, while

Rachuonyo and Homa Bay have lower PHC implementation levels. Views from community members and PHC implementers on priority NTDs were gathered using key informant interviews and focus group discussions. The status of prevailing PHC delivery services were assessed by use of checklists and policy document reviews. Differences in socio-cultural dynamics as related to health care delivery uptake in the study districts are discussed. Data from phase I will be used to design CDI strategies for the four study districts in Phase II for identified NTDs. The outcomes of this study can be used as lessons for implementation of practical integrated health interventions in other regions.

## 1069

### OPERATIONAL RESEARCH ON INTEGRATED NEGLECTED TROPICAL DISEASE (NTD) MAPPING: RESULTS FROM MALI AND SENEGAL

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There is increasing interest and funding for integrated approached to NTD programs with preventive chemoprophylaxis. To effectively implement these programs, integrated tools are currently being developed. CDC developed an integrated mapping protocol to help Ministries of Health decide where public health interventions for lymphatic filariasis (LF), trachoma, schistosomiasis and soil-transmitted helminths (STH) are needed. To validate the protocol, the integrated methodology and the current WHO methodologies were implemented in 1 district each in Mali and Senegal. The outcomes evaluated are the prevalence results and its public health intervention implications, survey costs and feasibility.

Both methodologies assessed the WHO-recommended indicators, with the specified age groups, but the sampling frame for the integrated methodology was adapted to make mapping more resource-efficient. The integrated methodology surveyed 2 villages in each sub-district: 1 village was selected randomly; the second was selected based on high suspected schistosomiasis prevalence. In Mali, 1,898 persons, including 900 children (1-9 years), in 18 villages were surveyed for the integrated methodology and 4,479 persons, including 2,738 children (1-9 years) for the WHO methodology, a total of 6,377 persons. Both methodologies indicated no need for mass treatment (MDA) in the surveyed district for trachoma (2.1%, 4.7% TF, respectively) and STH (0%, 0%) and a need for MDA with ivermectin for LF (7%). The integrated methodology indicated 4 sub-districts in need of MDA for schistosomiasis for school-aged children and 5 sub-districts in need of MDA for the whole population. The WHO methodology indicated MDA for school-aged children in the entire area. Using the integrated methodology resulted in a 29% overall cost savings ($7,468 vs $10,539). The integrated methodology used resources more efficiently in the areas of transport, survey time, and teams. Data in Senegal will be collected in June and also will be presented. The new integrated mapping tool could facilitate the beginning and scaling-up of programs by reducing the human and financial resources needed to gather evidence for deciding if a public health intervention is warranted.

## 1070

### THE DEVELOPMENT OF A SPECIMEN SPARING MULTI-CHANNELED BEADED ASSAY TO DETECT IGG4 ANTIBODIES TO *SCHISTOSOMA HAEMATOBIUM*, HOOKWORM AND FILARIAL INFECTIONS IN A POPULATION ON THE COAST OF KENYA

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To study the combined negative health impact of polyparasitism we developed a novel, diagnostic multi-channeled fluorescent antibody detection assay that simultaneously detects multiple parasitic infections. This assay was developed as part of a study of polyparasitism in coastal Kenya. As there is low cross-reactivity with IgG4, IgG4 responses to *Brugia malayi* antigen (BMA), *S. haematobium* soluble worm antigenic preparation (SWAP), and hookworm excretory/secretory proteins (ESP) were used to detect infections with *Wuchecheria bancrofti*, *Schistosoma haematobium*, and *Necator americanus*, respectively. These responses were assessed using a high throughput bead-based platform (Bioplex, Bio-rad, Hercules, CA). The antigens were coupled to beads, each with a unique dye allowing for automated discrimination of fluorescence. Pooled serum from areas endemic for these infections were used for optimization. With each well containing beads for all three helminthes, diluted serum was incubated with the beads and the RPE conjugated anti-human IgG4 (Southern Biotech) and the plates analyzed using the Bioplex. Due to the low output seen with ESP, beads will be linked to biotin and then coupled to ESP. Cutoff values were set using sera negative for the infections. Standard curves to determine the optimal dilutions were created using a serial dilution of the serum pool. Unlike the SWAP and BMA beads, the ESP beads did not fluoresce significantly with anti-IgG4 secondary antibody despite the detection of anti-ESP IgG4 in the serum with ELISA. Conjugating biotin to the beads will allow for the retention of the native conformation of the ESP and the appropriate binding to serum IgG4. We believe that this type of serologic testing will increase sensitivity of parasite diagnosis with better community participation, providing a better survey of the prevalence of co-infection in this area while offering a specimen sparing method of detection. Given the benefits of this assay and increasing interest in polyparasitism, this could provide a model for serological surveys in future studies.

## 1071

### NATIONWIDE INTEGRATED MAPPING OF THREE NEGLECTED TROPICAL DISEASES IN TOGO

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The Ministry of Health of Togo (MoH) has been working toward integrated control of neglected tropical diseases (NTDs) since 2003. In preparation for nationwide integrated MDA for NTDs in Togo, the MoH and partners conducted an integrated prevalence survey for schistosomiasis, soil transmitted helminthiasis (STH) and trachoma in all districts outside Lomé. Sampling was based on a new, integrated approach developed by the Centers for Disease Control and Prevention. Two villages in each of the 549 peripheral health units (PHU) in 29 of Togo's 35 districts (1096 villages total) were selected based on proximity to water and anticipated high prevalence of schistosomiasis. Trachoma was included for 14 districts. In each village 15 school children age 6 to 9 years were recruited; an

additional 35 children age 1 to 5 years were surveyed for trachoma. Informed consent was obtained. An MoH team collected stool and urine from the children and conducted an eye examination. Urine was tested for blood using a dipstick. Stool was tested for *S. mansoni* and STH using the Kato-Katz method. Over 6 weeks starting in October 2009, 16,440 children were tested for schistosomiasis and STH; 25,000 children were examined for trachoma. At the PHU level, the level planned for MDA implementation for schistosomiasis, the prevalence of *S. haematobium and S. mansoni* ranged from 0 to 100% and 0 to 93%, respectively (national averages: 20% and 3%). The prevalence of STH at the district level ranged from 5% to 70% (national average 32%). Hookworm accounted for 99% of STH detected. The prevalence of trachomatous follicular inflammation, the indicator of active trachoma, ranged from 0.5 to 11.3%. In conclusion, this national, integrated prevalence survey for three NTDs in Togo proved practical and efficient. Integrated MDA in each PHU will target the diseases and population as indicated by the local prevalence of each infection and WHO guidelines. Conducting prevalence mapping at the PHU level allows for focal distribution of preventive chemotherapy, reducing the likelihood of both over- and under-treatment of targeted populations compared to district level MDA implementation, and is more efficient and cost-saving than village level implementation.

## 1072

### THE AMAZONIC NEMATODE *STRONGYLOIDES* AND THE ANDEAN FLUKE *FASCIOLA*: THE NEED FOR TAILORING CONTROL PROGRAMS

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*Strongyloides stercoralis* infection is a threat in HTLV-I co-infected people (highly endemic in Peru) or in people receiving mainly chemotherapy or steroids. *Fasciola hepatica* infection is able to cause significant hepatic morbidity in people from endemic areas, vegetarians or frequent travelers. Objectives of this study were to update current prevalence rates and reported cases of *S. stercoralis* and *F. hepatica* infections in Peru and to describe the most sensitive diagnostic tests performed in these studies. The inclusion criteria included studies originated in Peru from January 2000 to April 2010 and published in the following databases: MEDLINE, LILACS, SCIELO Peru and LIPECS (Peruvian Literature in Health Sciences). For searching criteria, the following keywords were used: Strongyloides, Strongyloidiasis, Fasciola, Fascioliasis and Peru. A total of 1362 subjects from 52 studies were reported with *S. stercoralis* infection (prevalence rates: 0.3-39%) whereas 1136 subjects from 33 studies were reported with *F. hepatica* infection (0.2-27%). The Lumbreras' Rapid Sedimentation Technique (LRST) was the most effective coprological test for detection of Fasciola eggs; whereas the agar plate, Dancescu culture and the Modified Baermann's Method (MBM) had the highest sensitivity for detection of *Strongyloides* larvae. Strongyloides geographic distribution is mostly in the Amazonic region whereas Fasciola is largely present in the Andean Region. Control programs should pay attention on the distribution of specific hepatointestinal parasites. *Strongyloides* is mostly present in the Amazonic region, requires agar plate for prompt diagnosis and ivermectin is the treatment of choice. Fascioliasis is present throughout the Andean Region, LRST is the coprological test of choice and the most effective treatment is triclabendazole. This is an example of how difficult it can be to approach a national program control of gastrointestinal parasitic infections since two common prevalent parasites have opposite geographic distribution, require especial diagnostic tests and different treatments.

## 1073

### NEGLECTED GEOHELMINTHIASIS OF PERU IN THE NEW MILLENNIUM

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Infections by intestinal parasites cause significant morbidity including malnutrition, growth retardation and cognitive impairment. Helminthiasis is the fourth cause of outpatient visits in Peru; approximately 1 million cases were seen in 2008 according to the Peruvian Ministry of Health. The objective of this study was to update the current prevalence rates of intestinal parasites in Peru. The inclusion criteria included the studies originated in Peru from January 2000 to April 2010 and published in data bases such as MEDLINE, LILACS (Latin American Literature), SCIELO Peru and LIPECS (Peruvian Literature in Health Sciences). For searching criteria, the following keywords in Spanish and English were used alone or in combination: parasites, intestinal parasites, helminths, and Peru. A total of 101 studies met the inclusion criteria. Out of the 43,803 subjects who were examined in these studies, 55% of them were infected with at least one parasite (n=24,266). The most common helminths found were: *Ascaris lumbricoides* 15% (n=4894; range of prevalence 0.6-82%), *Trichuris trichiura* 14% (n=3018; 0.2-82%) and hookworm 13% (n=1686; 0.1-73%). Fifty-five percent of the studies used at least one sedimentation technique for stool examination. The most common protozoa was *Giardia lamblia* 24% (n=6936; 3-44%). The age group mostly affected was young people below 20 years old and the highest prevalence rates were present in the poorest and most forgotten communities. The highest prevalence rates in Peru in the last years are present in regions with poor health access and on severe poverty. Further studies are warranted to measure the impact of economic, simple and highly sensitive sedimentation techniques in combination with targeted massive population treatments. This is an example of how little we know about a very common public health problem in a developing country which has been forgotten even at the beginning of this new Millennium.

## 1074

### *TRYPANOSOMA CRUZI* I GENOTYPES IN DIFFERENT GEOGRAPHIC REGIONS AND TRANSMISSION CYCLES BASED ON A MICROSATELLITE MOTIF OF THE INTERGENIC SPACER OF SPLICED LEADER GENES

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The intergenic region of spliced-leader (SL-IR) genes from 105 *Trypanosoma cruzi* I infected biological samples, culture isolates and stocks from 11 endemic countries, from Argentina to USA, were characterised, allowing identification of 76 genotypes with 54 polymorphic sites from 123 aligned sequences. On the basis of the microsatellite motif proposed by Herrera et al. (2007) to define four haplotypes in Colombia, we could classify these genotypes into four distinct Tc I SL-IR groups, three corresponding to the former haplotypes Ia (11 genotypes), Ib (11 genotypes) and Id (35 genotypes); and one novel group, Ie (19 genotypes). Tc Ia was associated with domestic cycles in Southern and Northern South America and sylvatic cycles in Central and North America. Tc Ib was found in all transmission cycles from Colombia. Tc Id was identified in all transmission cycles from Argentina and Colombia, including Chagas cardiomyopathy patients, sylvatic Brazilian samples and human cases from French Guiana, Panama and Venezuela. Tc Ie gathered five samples from domestic *Triatoma infestans* from Northern Argentina, nine samples from wild *Mepraia spinolai* and *Mepraia gajardoi* and two chagasic patients from Chile and one from a Bolivian patient with chagasic reactivation. Mixed infections by Tc Ia + Tc Id, Tc Ia + Tc Ie and Tc Id + Tc Ie were detected in vector faeces and isolates from human and vector samples. In addition, Tc Ia and Tc Id were identified at different tissues from a heart transplanted Chagas cardiomyopathy patient with reactivation, denoting histotropism. *T. cruzi* I SL-IR genotypes from parasites infecting *Triatoma gerstaeckeri* and *Didelphis virginiana* from USA, *T. infestans* from Paraguay, *Rhodnius nasutus* and *Rhodnius neglectus* from Brazil and *M. spinolai* and *M. gajardoi* from Chile are described for the first time.

## 1075

### BOVINE ANTIBODY RESPONSE DIRECTED AGAINST *GLOSSINA* SALIVA: AN EPIDEMIOLOGIC MARKER OF CATTLE EXPOSURE TO TSETSE BITES

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Our objective is to develop a sero-epidemiological tool to measure the exposure of cattle to tsetse bites based on the host antibody response directed against *Glossina* total salivary antigens. The anti-saliva (IgG) response against *Glossina palpalis gambiensis* saliva was assessed by ELISA-indirect on 102 bovine sera from Burkina Faso: 48 were sedentary bovine from a tsetse free area (North) and 54 were from a tsetse infested area (South-West). High anti-saliva responses were detected in cows from the tsetse infested area. In these animals, the anti-saliva response was significantly higher during the hot dry season (p=0.0004) when animals are the most exposed to *Glossina* bites when watering at gallery forests along permanent streams. Furthermore, there was a strong positive association between the anti-saliva response and the risk of being infected by trypanosomes (p=0.004). These results show that the anti-saliva response may be an interesting marker of exposure of cattle to tsetse bites. However, some animals from the tsetse free area had also elevated anti-saliva responses, suggesting the existence of immune cross-reactivity with salivary proteins from other hematophagous arthropods. We have carried out experimental exposure of cows to the bite by different hematophagous vectors in order to further evaluate the use of *Glossina* total salivary antigens as marker of exposure. Good dynamic of apparition and disappearance of anti-saliva antibodies were observed in animals exposed to the different *Glossina* species whereas some cross reaction with the saliva of horse-flies are suspected. In perspectives, we want to identify and to synthesize tsetse saliva specific antigens in order to develop a more specific and standardized tool. This tool could be used in african trypanomosis endemic areas to (i) identify highly exposed herds toward which vector control should be directed to and to (ii) assess the efficiency of entomological control measures.

## 1076

### PREVALENCE OF HAEMO AND ECTOPASRASITES IN CAMELS SLAUGHTERED AT MAIDUGURI METROPOLITAN ABATTOIR, BORNO STATE, NIGERIA

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*Trypanosoma evansi* is the main cause of trypanosomiasis (Surra) in camels. Camels (*Camelus dromedarius*) are a popular source of protein next to beef in Northern Nigeria. It has also been known to be less infected with parasites. To determine the prevalence of hemo and ectoparasites in camels, two hundred camels (*C. dromedarius*) were sampled at the Maiduguri metropolitan abattoir in northern Nigeria, West Africa. Six of these were infected with *T. evansi,* and one was infected with *Babesia.* Also Eighty-three percent of these animals were infested by ectoparasites including ticks. Even though humans are not typically infected with *T. evansi,* there have been case reports of such infections in farmers. This preliminary report suggests a need for further research in this area. Furthermore trypanosomiasis in camels results in huge economic loss for farmers, and like their cattle counterparts camel farmers need to be educated about proper husbandry and disease prevention.

## 1077

### SMALL MAMMAL DIVERSITY AND *LEISHMANIA* INFECTION ACROSS A FOREST COVER GRADIENT IN SOUTHERN COSTA RICA

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Anthropogenic activities have transformed the landscape leading to gradients of forest cover worldwide. These fragmented and heterogeneous landscape patterns have been associated with the emergence and transmission of Cutaneous Leishmaniasis in Southern Costa Rica. One possible mechanism behind the emergence of this neglected tropical disease is the change in the biodiversity and abundance of mammal species, that can serve as reservoirs of Leishmania parasites, and that is mediated by concurrent changes in the landscape. Here, we present results of six months of small mammal species sampling using the advanced distance sampling, a new method that optimizes the sampling effort to estimate demographic parameters from wildlife populations employed along several plots across a forest cover gradient in southern Costa Rica. We think the use of this new sampling methodology can improve our understanding of the transmission dynamics of this disease by providing demographic information necessary to understand the eco-epidemiology of this pathogen in the reservoirs.

## 1078

### EPIDEMIOLOGY OF AMERICAN TEGUMENTAR LEISHMANIASIS IN THE XAKRIABÁ INDIGENOUS COMMUNITY, STATE OF MINAS GERAIS, SOUTHEAST BRAZIL: A CROSS SECTIONAL STUDY

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American Tegumentar Leishmaniasis (ATL) is a serious health problem in the Xakriabá indigenous community, in the northeast of Minas Gerais, Brazil. Objectives: to estimate the prevalence and identify possible risk factors of clinical and asymptomatic ATL within the Xakriabá. A survey was carried out in two villages with a higher number of recent human cases of ATL. A sample of the population was interviewed using pre-coded questionnaires investigating the demographic characteristics, socio-economic status, personal and cultural habits, characteristics of domiciles and neighborhoods. Presences of reservoirs and phlebotomies species were also investigated. The diagnoses were carried out using clinical evaluations, skin tests, parasitological (direct examination and culture) and molecular techniques (PCR-RFLP). Immunological profiles are also being constructed. A sample of 164 individuals was studied. The mean age was 22.5 ±16.6 years and females constituted 59% of the sample. Within this low socio-economic community, 65% had only elementary school education levels. Most participants (85%) reported daily contact with the forest. The majority usually burn manure in their houses to protect them against insect bites. Among the participants, 46% reported that a household member has had skin lesions. Only 16 (9.8%) of interviewees reported knowledge of the sandfly, with 15 mentioning either the typical bite with small red points, or the style of flying in short jumps. When photos were shown to the interviewees, 75.3% reported that they had previously seen the disease. Eleven clinical cases were identified: 9 were confirmed by parasitological examinations as *Leishmania braziliensis* and 2 were classified as clinical-epidemiological cases and were cured after treatment. The prevalence was estimated at 6.7% (IC 95% 3.6-11.4). Among the 11 cases, 8 (72.7%) presented scars (probably relapses or reinfections). The prevalence of asymptomatic infection was 18.5% (IC 95% 12.9-25.3). The ages of asymptomatic individuals were significantly higher (P<0.001) than negative individuals. There was no sex difference among asymptomatic, clinical cases, and negative individuals. Other characteristics and adjustments are being investigated. Conclusion: this study confirms the high prevalence of ATL and asymptomatic infection and the high occurrence of relapses and reinfection in the Xakriabá community

## 1079

### INDUCTION OF ATHEROMATOUS PLAQUES IN WISTAR RATS CHRONICALLY INFECTED WITH *TRYPANOSOMA CRUZI* AND FED AD LIBITUM WITH DIET RICH IN FATS

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This work is focused on the induction of atheromatous plaques in male albino rats Wistar (*Rattus norvegicus*), chronically infected with *Trypanosoma cruzi* and fed *ad libitum* with diet rich in fats of vegetal origin, during three months. The chronic infection detected by serological and parasitological assays, revealed the presence of antibodies IgG anti-*T. cruzi* and the absence of parasitemias. The diet rich in fats produced in the group of infected rats (A) and the group of healthy rats (C) a significant increase in the weight (P<0.05), in comparison with the control group of infected rats (B) and the group of healthy rats (D), fed with a normal diet. The histopathological study of sections of aorta artery of rats of the group A (infected/diet fats), showed abundants lipid deposits, inflammatory processes (vasculitis) and atheromatous plaques in development. The sections of the heart and skeletal muscle showed pictures of a myocarditis and myositis with features of chronic tissue without parasitism. The immunoistochemestry assay applied to the cuts of artery, heart and skeletal muscle of infected rats A (diet/fast) and B (normal/diet), showed abundants antigen deposits. In conclusion, the rat chronically infected with *T. cruzi* and fed with a diet rich in fats, have a main propensity to develop atheromatous plaques. The results showed that a hyperlipidic diet is a risk factor for the development of atheromatous disease in individuals with Chagas´disease.

## 1080

### CHAGAS' DISEASE AMONG CHILDREN AND MOTHERS IN MAYAN RURAL COMMUNITIES: UTILIZING PDA/GPS UNITS FOR HOUSEHOLD SURVEYS

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The protozoan parasite *Trypanozoma cruzi* is the causal agent of Chagas' disease, a major vector-borne disease in Latin America. We determined the prevalence of *T. cruzi* infection in children (0-12 years old) and their mothers in two communities (Sudzal and Teya) in Yucatán, Mexico by conducting a household survey of mothers and their children. We utilized a suite of mobile technologies (PDA/GPS) to geo-reference 371 households and to collect the survey response data. Within the households, 685 children and 390 mothers were surveyed, which represented a 94% acceptance and participation rate. The ages of the children surveyed were from 0-12 years old; the ages of the mothers surveyed were from 15-59 years old. *T. cruzi* seropositivity was determined with a rapid test (Stat-Pack) and an ELISA assay (Wiener recombinant ELISA) and confirmed by the Institute of Reference and Diagnostic Epidemiology in Mexico, which used two additional diagnostic tests. Participants were considered seropositive when a sample was seroreactive to two or more tests. The

prevalence rates among children were 0.7% (2/193) in Sudzal and 0.3% (1/392) in Teya; the seroprevalence rates among their mothers were 4.4% (7/160) in Sudzal and 0.9% (2/230) in Teya. The higher seroprevalence of *T. cruzi* infection in Sudzal may be due to differences in sociodemographic, economic, or entomologic factors between these communities. These results draw attention to the need for Chagas' disease prevention programs in the region.

## 1081

### IDENTIFICATION OF TRYPANOSOMES ISOLATED FROM HUMANS AND ANIMAL RESERVOIRS IN THE *TRYPANOSOMA BRUCEI GAMBIENSE-T.B. RHODESIENSE* INTERFACE DISTRICTS OF NORTHERN UGANDA

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Human African Trypanosomiasis (HAT) due to *Trypanosoma brucei gambiense* (chronic) or *T. b. rhodesiense* (acute) is a major public health problem in rural Uganda. Continued northward spread of *T. b. rhodesiense* from the Southeast towards *T. b. gambiense* endemic districts (Northwest) is feared to complicate diagnosis and treatment if the 2 diseases co-exist. In order to enable the National HAT programme institute informed control measures, we aimed to characterize trypanosomes from patients and domestic animal reservoirs in the interface districts of Northern Uganda. We carried out surveys in Lira, Dokolo, and Amuru districts. Of 43 persons found positive by the Card Agglutination Test for Trypanosomiasis (CATT) in Lira, 1 was confirmed parasite positive after Haematocrit Centrifugation technique (HCT). Analysis on the 43 CATT positive blood spots on FTA cards by PCR with TBR, SRA and TgsGP primers revealed *T. brucei* signals in 23, *T. b. rhodesiense* in 2; all were *T. b. gambiense* negative. Blood from 20 suspects (*T. brucei* signals only) was inoculated in mice. None became positive, indicating probable exposure to *T. brucei brucei* (non-human infective). We also tested samples passively collected from Lwala hospital that serves Dokolo and Lira, so far all are *T. b. rhodesiense*. From Amuru district in the north (about 100Km from the Lira sites), 1 person was found positive with *T. b. gambiense*. Of 1,782 domestic animals (1,713 cattle, 22 pigs, 19 goats, 18 dogs and 10 sheep) screened in Dokolo, 99 (96 cattle, 2 sheep and 1 pig) were confirmed parasite positive by HCT. Amplification of the rDNA Internal Transcribed Spacer (ITS) in 40 samples from cattle demonstrated *T. brucei* (a complex including the human infective subspecies) in 18, *T. congolense* in 11 and *T. vivax* in 6 animals. This preliminary observation that *T. brucei* (the least pathogenic among animal trypanosomes) is the most prevalent implies that farmers may not readily seek intervention, leading to an unchecked animal reservoir. Indeed 16 of the *T. brucei* were found to be *T. b. rhodesiense* by SRA-PCR.

## 1082

### *TRYPANOSOMA BRUCEI* PARASITES FAVOR THEIR TRANSMISSION BY MODIFYING THE TSETSE FLY SALIVARY COMPOSITION

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Tsetse flies are the notorious transmitters of African trypanosomiasis, a disease caused by the *Trypanosoma* parasite that affects humans and livestock on the African continent. Here, the infected fly/host contact frequency is a key determinant of the transmission dynamics. As an obligate blood feeder, tsetse flies rely on their complex salivary potion to inhibit host haemostatic reactions ensuring an efficient feeding. The results of this experimental study suggest that the parasite might promote its transmission through manipulation of the tsetse feeding behavior by modifying the saliva composition. Indeed, salivary gland *Trypanosoma brucei*-infected flies display a significantly prolonged feeding time, thereby enhancing the likelihood of infecting multiple hosts during the process of a single blood meal cycle. Comparison of the two major anti-haemostatic activities i.e. anti-platelet aggregation and anti-coagulation activity in these flies versus non-infected tsetse flies demonstrates a significant suppression of these activities as a result of the trypanosome-infection status. This effect was mainly related to the parasite-induced reduction in salivary gland gene transcription, resulting in a strong decrease in protein content and related biological activities. Additionally, the anti-thrombin activity and inhibition of thrombin-induced coagulation was even more severely hampered as a result of the trypanosome infection. Indeed, while naive tsetse saliva strongly inhibited human thrombin activity and thrombin-induced blood coagulation, saliva from *T. brucei*-infected flies showed a significantly enhanced thrombinase activity resulting in a far less potent anti-coagulation activity. These data clearly provide evidence for a trypanosome-mediated modification of the tsetse salivary composition that results in a drastically reduced anti-haemostatic potential and a hampered feeding performance which could lead to an increase of the vector/host contact and parasite transmission in field conditions.

## 1083

### A HIGH-THROUGHPUT *IN VITRO* SCREEN TO IDENTIFY INHIBITORS OF THE *TRYPANOSOMA BRUCEI* TRYPANANTHIONE SYNTHETHASE ACTIVITY

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The protozoan parasite, *Trypanasoma brucei* is the primary causative agent for HAT (Human African Trypanasomiasis) which results in >50,000 deaths on a yearly basis in sub-Saharan Africa. Current therapies require lengthy hospitalization at a prohibitive cost and are very limited in scope due to toxic side effects and acquired drug resistance. The thiol-redox pathway plays an important role in the defense against chemical and oxidative stress and has been determined to be essential for the survival of *T. brucei*. Due to the absence of this particular biochemical pathway in man, the enzymes within this pathway, including trypanathione synthetase (TryS), represent excellent novel targets for HAT drug discovery efforts. A TryS activity assay was developed at the University of Dundee and a high-throughput screen of 215,000 compounds at 10 uM was undertaken at Genzyme Corporation in an effort to identify inhibitors of the *Tb*TryS. Following hit confirmation, several compounds were chosen to undergo IC50 potency determinations, and three novel series of active compounds were identified around which a medicinal chemistry program could be initiated. The activity of these compounds in a cultured *T. brucei* parasite assay will be determined in an effort to establish a correlation between *in -vitro* activity and *in- vivo* potency.

## 1084

### TWO HIGHLY CONSERVED SECRETORY NUCLEASES FACILITATE PURINE SALVAGE IN *LEISHMANIA MEXICANA*

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All *Leishmania spp* are purine auxotrophs, i.e. they must salvage these molecules from both their insect/mammalian hosts to fulfill their metabolic needs. Since purines are critical to leishmanial survival, we deemed it important to elucidate the mechanisms used for such acquisition. In that regard, we identified two 35kDa secretory nucleases *Lmex*NUCs-1 and -2 in *Leishmania mexicana* which are differentially transcribed and translated through the parasite's life cycle (Am>>Prom). The two 951bp nuclease ORFs share 95% nucleotide homology and possess the five

structural motifs characteristic of the P1/S1 family of fungal nucleases. To understand the biological implications of these two nucleases, we over-expressed *Lmex*NUCs-1 and -2 in *L.mexicana*. Using molecular and biochemical techniques, we demonstrated that *Lmex*NUCs-1 and -2 chimeric enzymes were expressed and secreted/released constitutively by both *L.mexicana* promastigote and amastigote developmental forms. Further, we found that the chimeric and wild type enzymes were functionally active in hydrolyzing a variety of natural and synthetic substrates (i.e. Poly-A, -U, -I, RNA, ssDNA and dsDNA). Such nuclease activity required a metal co-factor and was inhibited by DTT reduction. In addition, we showed that *Lmex*NUCs-1 and -2 were actively synthesized by amastigotes within infected mouse macrophages. Our cumulative results suggest that *Lmex*NUCs-1 and -2 play important role(s) in facilitating the growth, development and survival of this human pathogen.

## 1085

### FUNCTIONAL, MORPHOLOGICAL AND METABOLIC EVALUATION OF MICE ACUTELY INFECTED WITH THREE DIFFERENT STRAINS OF *TRYPANOSOMA CRUZI*

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Strains of *Trypanosoma cruzi* are multiclonal populations that can be classified in groups or genotypes, differing in pathogenicity, virulence, and histotropism. In this study, functional, morphological and metabolic evaluation of mice infected with three strains of *T. cruzi* was performed in the peak of mortality of the animals. CD1 mice infected with 5x104 of the Brazil strain or C57Bl/6 mice infected with 1x103 of the Y strain or C57Bl/6 mice infected with 1x103 of the Tulahuen strain were used. Parasitemia, histopathology, echocardiography (Echo), magnetic resonance imaging (MRI), microPET and body composition (BC) were evaluated. 60% of CD1 mice infected with the Brazil strain died 28-32 dpi, when the parasitemia was decreasing. They presented generalized edema characterized by increased amount of water and fluids and decreased fat at BC analysis. The myocarditis was more evident in the right ventricle (RV) than in the left ventricle (LV). The RV was dilated and the cardiac frequency was decreased in the Echo. The cardiac metabolism was changed from fatty acid to carbohydrate oxidation. 100% of C57Bl/6 mice infected with the Tulahuen strain died 18-24 dpi when the parasitemia was still increasing. They presented increased amount of water and fluids and decreased fat at BC analysis. The myocarditis was present in both ventricles. The RV was dilated and there was no alteration in the Echo. The cardiac metabolism was changed from fatty acid to carbohydrate oxidation. 100% of C57Bl/6 mice infected with the Y strain died 16-23 dpi when the parasitemia was decreasing. They presented increased amount of water and decreased fat at BC analysis. The myocarditis was present in both ventricles. The RV was dilated and the wall thickness of RV and LV was increased. There was no alteration in the Echo. The cardiac metabolism was changed from fatty acid to carbohydrate oxidation. The results corroborate that the genetic differences between the *T. cruzi* strains correlate with their tissue tropism and can help to explain the different findings.

## 1086

### IDENTIFICATION OF PROTEINS DIFFERENTIALLY EXPRESSED IN *LEISHMANIA (VIANNIA) BRAZILIENSIS* AND *L.(V.) PERUVIANA* BY TWO DIMENSIONAL GEL ELECTROPHORESIS

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The cellular and molecular basis that supports the progress from cutaneous to mucous Leishmaniasis (metastasis) is not well understood. *Leishmania (V.) peruviana* and *L.(V.) braziliensis* are two species so closely related that are almost indistinguishable when analysed at the DNA level. However, despite their proximity, *L.(V.) peruviana* only causes cutaneous Leishmaniasis, whereas *L.(V.) braziliensis* can as well disseminate to distant tissues ending in mucous leishmaniasis. For studying this event, a proteomic study was performed to identify proteins differentially expressed by these two species as candidate metastasis markers. Promastigotes of LC2043 (*L.(V.)braziliensis*) and HB86 (*L.(V.)peruviana*) strains were lysed by freeze and thaw cycles and 500ug of soluble proteins were applied in 18cm, non-linear strips for isoelectrofocusing. After, they were separated according their molecular weight by 2DE electrophoresis and gels were analyzed with ImageMaster™ 2D Platinum v7.0. Landmark proteins were detected and gels images calibrated according to their isoelectric point (*pI*) and molecular weight (*Mr)*. While using strips with pI from 3-10, results showed that similar to *L.(V.)braziliensis (1)*, most of the spots from *L.(V.) peruviana* were in the 4-7 *pI* range. From 2 independent experiments, approximately 396 spots were detected in the *L.(V) peruviana* samples, with proteins predominantly from 22 to 80 kD. Comparison of the two proteomes revealed one protein specifically associated with LC2043 mucosal strain. Interestingly, these spot was not present in the HB86 cutaneous strain proteome. On the contrary, other spot (*pI* 7.6) was exclusively expressed in the *L.(V.) peruviana* proteome. In this study, the first *L.(V.) peruviana* protein map was obtained. Furthermore, we have found 2 spots differentially expressed in the *L.(V.) braziliensis* and *L.(V.) peruviana* proteomes that might be markers associated to metastasis. Therefore, it is of pivotal importance to characterize these proteins by mass spectrometry (MALDITOF) and test them on their potential of being metastasis markers.

## 1087

### ALGORITHM PERFORMANCE ASSESSMENT OF PCR AND PCR-RFLP ASSAYS FOR *LEISHMANIA VIANNIA* SPECIES IDENTIFICATION

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*Leishmania* species identification is currently performed based on PCR assays because their high sensibility made them useful on clinical samples. Different targets are used to identify *Viannia* species but there is no consensus methodology described. We present an algorithm based on a three target PCR strategy that can discriminate specifically between *L. peruviana, L. braziliensis* and *L. guyanensis*. The algorithm integrates three different targets previously reported for *Viannia* species identification: mannose phosphate isomerase (MPI) PCR that can identify specifically *L. (V.) peruviana*, cystein proteinase B (CPB) PCR-RFLP that can identify *L. (V.) braziliensis* and heat shock protein 70 kDA (HSP70) PCR-RFLP that differentiates specifically *L. (V.) guyanensis*. We analyze field isolates and clinical samples from patients that yield intense bands in kDNA diagnostic PCR. The samples analyzed include aspirates, scrapings and filter paper. Concerning to field isolates, we successfully identified *Leishmania* species

in 79 out of 80. The species founded were mainly *L. peruviana* (23)*, L. braziliensis* (41) and *L. guyanensis* (14). Only one isolate revealed evidence that suggest that it correspond to a *L. braziliensis/L. peruviana* hybrid. Sensibility of the targets demonstrates that MPI PCR has the highest while lower values were seen in HSP70 PCR. Additionally, when clinical samples from 12 patients were analyzed,analyzed; we successfully identify species in all of them. However, results vary based on different sensibility raised with different types of clinical samples. In general, filter paper reaches higher sensibility values. In conclusion, our algorithm is specific for *Leishmania Viannia* species identification. It can be used to estimate cost of species identification according known species prevalence in different endemic areas, with consequent improvement of hich can be useful to improve leishmaniasis healthcare decision makingpolicies. A consensus strategy as our algorithm is potentially useful for clinicians for decision-making in terms of treatment outcome and patient management.

## 1088

### ASSESSING GENE STATUS AND EXPRESSION PROFILE OF AQUAGLYCEROPORIN, ABC TRANSPORTER MRPA, ORNITHINE DECARBOXYLAE AND Γ-GLUTAMYLCYSTEINE SYNTHETASE IN ANTIMONY-SUSCEPTIBLE AND RESISTANT CLINICAL ISOLATES OF *LEISHMANIA DONOVANI* FROM INDIA

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Clinical resistance to pentavalent antimonials is interplay between uptake, efflux and sequestration in Leishmania but resistance to this drug in the field isolates is not clearly understood. To address this question, in the present study, we have characterized clinical isolates from India and report that diverse mechanisms of resistance are operative in these isolates. In the present study, we examined the role of Aquaglyceroporin (AQP1), ABC transporter (MRPA), ornithine decarboxylase (ODC) and γ-glutamylcysteine synthetase (γ-GCS) genes as possible biomarkers for monitoring antimonial resistance in Indian leishmaniasis. Aquaglyceroporins (AQPs) have been shown to facilitate uptake of trivalent metalloids. The ABC tranporter gene MRPA confers resistance by sequestration of metal-thiol conjugate. Ornithine decarboxylase (a rate limiting enzyme in polyamine biosynthesis), and γ--glutamylcysteine synthetase (a rate limiting enzyme in glutathione biosynthesis) are two building blocks of the main cellular thiol trypanothione. Susceptibility to trivalent antimony as determined *in vitro* with intracellular amastigotes from both visceral (VL) and post-kala-azar dermal leishmaniasis (PKDL) patients correlated well with the clinical response. Reduced accumulation of SbIII correlated, with a few exceptions, to downregulation of AQP1 RNA as determined by real-time PCR in resistant isolates. Transfection of AQP1 gene in a SAG-resistant field isolate conferred sensitivity to the resistant isolate. However, increased expression of MRPA by real-time PCR was observed in resistant isolates. Transfection of MRPA in a sensitive isolate indeed conferred resistance to SAG in intracellular parasites. Cysteine and glutathione levels were increased in the SAG resistant isolates. Ornithine decarboxylase and γ--glutamylcysteine synthetase were found upregulated in all of the resistant isolates. Transfection of γ-GCS in a sensitive isolate led to 3 fold resistances to SAG in intracellular amastigotes, thereby confirming the role of γ-GCS as one of the factors involved in SAG resistance. We also found that the ODC overexpressors exhibited significant resistance to Pentostam compared to the wild type cells (>8.8 fold). A variety of resistance mechanisms to SAG, most of them consistent with a model based on the study of resistance *in vitro*, were present in clinical isolates from the same geographical region.

## 1089

### DISCOVERY OF 2,4-DIAMINOPYRIMIDINES AS POTENT INHIBITORS OF *TRYPANOSOMA BRUCEI* AND IDENTIFICATION OF MOLECULAR TARGETS BY A CHEMICAL PROTEOMICS APPROACH

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Human African Trypanosomiasis (HAT) is a fatal disease caused by *Trypanosoma brucei spp*. There is a need for new treatments for HAT because current drugs are costly, difficult to administer and frequently toxic. We have identified 2,4-diaminopyrimidines that demonstrate potent *in vitro* activity against *T. brucei brucei* and the related trypanosomatids *Leishmania spp*. *In vitro* studies performed to characterize the relationship between killing of *T. brucei* and compound exposure demonstrate an early (9-12 hrs) onset of trypanocidal activity as shown by the inability of the parasites to generate ATP. Parasite commitment to death *in vitro* occurs with similar kinetics, even when compound is washed out following a short exposure. A representative 2,4-diaminopyrimidine cured an acute trypanosomiasis infection in mice when administered orally at 20 mg/kg twice daily for 4 days. To identify the molecular target(s) responsible for the mechanism of action of 2,4-diaminopyrimidines against trypanosomatids a representative analogue was immobilized on a solid matrix (sepharose) and used to isolate target proteins from parasite extracts. Mitogen-activated protein kinases (MAPKs) and cdc2-related kinases (CRKs) were identified as the major proteins that were specifically bound to the immobilized compound, suggesting their potential participation in the pharmacological effects of 2,4-diaminopyrimidines against trypanosomatid protozoan parasites. Because they exhibit a good preliminary *in vitro* and *in vivo* pharmacological profile, and they target essential kinases, 2,4-diaminopyrimidines represent a potential lead class of small molecules for development of novel treatments for HAT.

## 1090

### INFLUENCE OF MACROPHAGE IMMUNE RESPONSE TO *LEISHMANIA BRAZILIENSIS* IN THE PATHOGENESIS OF TEGUMENTARY LEISHMANIASIS

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Macrophages are preferentially infected by *Leishmania* and serve both as a site of parasite multiplication and also are involved in *Leishmania* killing. In contrast to the wide knowledge of T cell in human leishmaniasis, very little is known about macrophage behavior in human *Leishmania* infection. The current study describes the immune response of macrophages in cutaneous leishmaniasis (CL) and mucosal leishmaniasis (ML) patients, evaluating the chemokines/cytokines produced after *L. braziliensis* infection. We also evaluated the frequency of inflammatory monocytes (CD14+CD16++) in the peripheral blood and TNF-α production by these cells. PBMC-derived macrophages from 21 CL patients 11 ML patients and from14 healthy subjects (HS) were infected with *L. braziliensis* and the levels of CCL2, CCL3, CXCL8, CXCL9 and TNF-α production were measured in supernatants of cultures by ELISA. Expression of CD14, CD16 and TNF-α production in peripheral blood monocytes were analyzed by flow cytometry. CCL2 production was higher in ML than in HS, p=0.0005. The levels of CXCL9 were significantly higher in CL patients and in ML patients when compared with HS, p=0.0001. The levels of TNF-α produced by macrophages from ML and CL patients were also higher than those produced by macrophages from HS, p=0.002 and p= 0.017

respectively. The frequency of CD14+CD16++ monocytes was higher in patients with CL and ML than HS,p=0.01 and p=0.03 respectively. The TNF-α production by cells from CL patients stimulated with soluble leishmania antigen was higher in CD14+CD16++ than in CD14CD16- cells, p< 0.005.The observation that macrophages from tegumentary leishmaniasis patients produce high levels of inflammatory chemokines/cytokines and show a higher expression of CD16++ cells suggest that these cells may participate in the pathogenesis of this disease.

## 1091

### DETECTION AND PERSISTENCE OF HOST DNA IN BLOOD MEALS FROM *TRIATOMA INFESTANS* USING A NOVEL MOLECULAR METHODOLOGY

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*Trypanosoma cruzi*, the etiologic agent of Chagas disease, is transmitted by hematophagous reduviid bugs within the subfamily Triatominae. These vectors use a wide range of hosts as blood meal sources, and determining the feeding behaviors of such bugs is useful for ecological and epidemiological studies of *Trypanosoma cruzi*. A number of different approaches for blood meal analysis have been described previously, including serologic and molecular detection methodologies. In the current investigation, the temporal pattern of blood meal detection in *Triatoma infestans* was investigated using a novel molecular approach. Third and fourth instar, lab-reared *T. infestans* nymphs (n=20) were allowed to feed on the blood of one of eight animal species (*Canis familiaris*, *Cavia porcellus*, *Rattus norvegicus*, *Gallus gallus, Mus musculus domesticus*, *Sus scrofa*, *Felis catus*, and *Homo sapiens*). At 7, 14, 21, and 28 days post-feeding, the guts of five bugs were individually collected. The molecular technique used for host identification from vector blood meals was a heminested PCR using novel general mammalian and avian primers. Resulting amplicons were sequenced to determine the species of the experimental blood meal source. Findings indicated that host DNA could be detected up to 28 days post-feeding, and the species of the blood meal source could be differentiated at the same time point. The implications of this study are two-fold. First, unlike other molecular approaches that utilize species-specific primers, this method can be used in an area where the animal species and feeding behavior of the triatomines are unknown. Secondly, this method was able to detect host DNA after an extended period of time in the *T. infestans* gut, indicating the detection capabilities of this assay when used in a field ecological or epidemiological study.

## 1092

### DEVELOPMENT OF LUMINESCENCE-BASED *LEISHMANIA* INTRACELLULAR AMASTIGOTE ASSAY FOR DRUG SCREENING

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The requirement for discovering new anti-leishmanial therapeutics remains due to the less than optimal treatments currently available. Since amastigote screening assays most closely reflect the pathological life cycle of the form found in mammalian hosts, many researchers consider axenic or intracellular amastigotes to be the most relevant *in vitro* assay cell types for leishmanial drug discovery. In order to establish a high-throughput *Leishmania* intracellular amastigote assay for use in our cutaneous leishmaniasis drug discovery program, we created *L.braziliensis, L. major, L. panamensis,* and *L. tropica* luciferase-expressin transfectants. Basic assay requirements were established with a manual 96-well assay using *L. major*. The IC50s of a limited panel of known anti-leishmanial compounds were determined to test the validity of the assay. To optimize assay conditions, starting macrophage and promastigote numbers, various macrophage cell lines, assay duration, plate centrifugation, luciferin-d concentration, and time of luciferin addition were examined. Currently, the assay has been automated to 96- and 384-well assay formats with continued IC50 testing of an expanded drug panel. The luminescence-based intracellular *Leishmania* amastigote assay has demonstrated the assay robustness, throughput, and reproducibility lacking from previously described amastigote assays.

## 1093

### EFFECT OF HEAT SHOCK STRESS ON THE EXPRESSION OF THE VIRULENCE FACTOR GP63 IN *LEISHMANIA MAJOR*

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Leishmania parasites must survive and adapt to different environments, from the vector to the final host. Their gene expression is regulated at the post-transcriptional level, where the 3' UTR regions play a fundamental role. Heat stress affects mRNA stability in parasite heat shock proteins, nevertheless, this heat stress has not been assayed in virulence factors. *In vitro* drug testing is usually made at random, not understanding what processes are being disturbed by promising compounds until long lasting investigations. Then, standardized models for evaluation of drugs that possibly modify gene expression in parasites could be stablished. The objective of this study was to evaluate the effect of temperature in the gene expression of the surface protein GP63 in *Leishmania major*. According to GeneDB, there are 4 copies of gp63 in the *Leishmania major* genome, with main differences at the 3' UTR among them. Primers have been designed to discriminate between them at mRNA level. Promastigote cultures, both in logarithmic and stationary phase, have been exposed to 24, 34 and 37 ºC for 3 hours prior to RNA extraction. Reverse transcription was performed with OligodT to ensure post-transcriptional information, subsequently polymerase chain reaction (PCR) was performed. The PCR products were observed by agarose gel using ethidium bromide. No difference was noticed in expression of gp63 genes 1, 2 and 4 at different temperatures in both phases. However, the expression of gene number 3 shows trends of variation between logarithmic and stationary phases. Effect of temperature remains unclear in those phases for expression of gene number 3. Further investigations on factors modifying gp63 number 3 expression need to be pursued. An *in vitro* model for testing drugs and compounds that impair natural gene expression in Leishmania could be developed in this context.

## 1094

### *TRYPANOSOMA CRUZI* TRUNCATES APOLIPOPROTEIN-A1 IN HUMAN HDL AND POTENTIALLY RESULTS IN A HYPERFUNCTIONAL HDL PHENOTYPE

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Chagas disease (CD) is caused by the protozoan parasite, *Trypanosoma cruzi*. Endemic in Central and South America where ~17 million persons are infected, latent infections can persist for decades, causing terminal, cardiomyopathy in ~30% of subjects. Current diagnostic tests often cannot discriminate between disease stages, and as a result, patients are treated with chemotherapy regardless of the disease status. Side effects of chemotherapy include granulocytopenia, rash and peripheral neuropathy. New diagnostic strategies are urgently needed as are tools that permit clinicians to target therapy more effectively. According to previous observations, CD patients, even those who die from cardiac complications have a lower incidence rate of atherosclerosis. However, levels of HDL and Apo-A1 in CD patients are reported to be normal. Recently, our laboratory

discovered several novel biomarkers for CD using SELDI-TOF, as reported previously. This set of biomarkers may indicate the specific disease stages of individual patients and the risk of further disease progression. Most importantly, we have identified intact Apo-A1 as a negative biomarker for CD and several truncated forms of Apo-A1 as positive biomarkers for CD. Apo-A1 is the principle protein found in high density lipoprotein (HDL). We have demonstrated that the principal cystein protease of *T. cruzi* cleaves Apo-A1 in HDL as predicted by mass spectrometry data. We have also shown that HDL is collected onto the surface of the blood-stage form and is internalized into the target host cell by the parasite by immunofluorescent staining. We also confirmed that mice chronically infected with *T. cruzi* show similar patterns of Apo-A1 cleavage. As well, preliminary data suggest that HDL from CD infected mice is 20% better at cholesterol efflux than native HDL. We are currently working with a mice model with HDL depletion to investigate further the interaction between *T. cruzi* and host HDL and the impact of *T. cruzi* infection on host lipid homeostasis. Our unanticipated observations give unique insights into the potential protection against atherosclerosis in CD individuals.

## 1095

### *LEISHMANIA AETHIOPICA*; THE UNUSUAL ETIOLOGIC AGENT OF CUTANEOUS LEISHMANIASIS IN HO DISTRICT OF THE VOLTA REGION OF GHANA

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Leishmaniasis is a parasitic disease of significant public health importance. An outbreak of suspected cutaneous leishmaniasis (CL) was first seen in the Volta Region of Eastern Ghana in 1999, and has remained an endemic area ever since. To improve the level of understanding regarding leishmaniasis in West Africa, particularly in Ghana, there is a need to provide information for the management of the disease. The study focused on the identification of species of Leishmania parasites responsible for leishmaniasis infections reported in the Volta Region of Ghana. The Ho district, located in the middle zone of the Volta region in the south-eastern part of Ghana, was the study site. It borders on the east with Togo in the West African sub-region. Forty four samples were taken for the study. Skin scrapings were collected from the sites of active lesion(s). Primers P5 and P6, were used to amplify a fragment of ~1500 bp of the intergenic region between the ribosomal protein genes RPS7A and RPS7B on chromosome 1 and second primers P1 and P2, were used to amplify an internal fragment of ~1350bp in the nested PCR. Products obtained from the nested PCR were digested using MspI enzyme. The bands produced from some samples showed a match to the control sample L. aethiopica. PCR was shown to be a useful diagnostic tool for Ghanaian CL.

## 1096

### WIDESPREAD SEGMENTAL, FOCAL COPY NUMBER VARIATIONS (CNV) IN *TRYPANOSOMA CRUZI* STRAINS REVEALED BY ARRAY COMPARATIVE GENOMIC HYBRIDIZATION

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*Trypanosoma cruzi* is a protozoan parasite and the etiologic agent of Chagas disease, an important public health problem in Latin America. *T. cruzi* is diploid, almost exclusively asexual, and displays an extraordinarily diverse population structure both genetically and phenotypically. Yet, to date the genotypic diversity of *T. cruzi* and its relationship, if any, to biological diversity have not been studied at the whole genome level. In this study, we used whole genome oligonucleotide tiling arrays to compare gene content in biologically disparate *T. cruzi* strains by comparative genomic hybridization (CGH). We observed that *T. cruzi* strains display widespread and focal copy number variations (CNV) and a substantially greater level of diversity than can be adequately defined by the current genetic typing methods. CNV were found in both core genes and gene family-rich regions of the genome, although primarily within the latter. Moreover, our results suggest that there is a greater degree of chromosome resorting between *T. cruzi* strains than previously thought.

## 1097

### UNDERRATING MOSQUITOES AGAIN: SURVEILLANCE EVASION BY *PLASMODIUM FALCIPARUM* ANTIFOLATE DRUG RESISTANCE MUTANTS

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By definition, "malaria" was an initial underrating of mosquitoes, ascribing the disease to "bad air". Ironically, present-day surveillance for drug-resistant *Plasmodium falciparum* alleles is based on genotyping microscopy-positive human malaria infections and presumed epidemiologically representative. By examining *P. falciparum* antifolate resistance genotypes in both human and mosquito hosts, here we show evasion of surveillance by *P. falciparum* drug resistance mutants. Using spray catches, 796 predominantly *Anopheles* arabiensis vector mosquitoes were captured from sleeping rooms of a representative sample of 2279 human residents in the vicinity of Macha, Southern Zambia. The cross-sectional composition of *P. falciparum* DHFR polymorphisms in both human and mosquito infections was examined using PCR and allele-specific restriction enzyme digestion, including DNA sequencing confirmations. We found high levels of pyrimethamine resistance mutants in human *P. falciparum* infections, with nearly saturated S108N (92.7%) and considerably prevalent N51I (81.5%) and C59R (58.5%). In contrast, the odds of these mutants were up to 101-fold lower in the mosquito phase (OR [95% CI]: 101.3 [34.34 - 299.03], $p < 0.001$). Instead, mosquito infections exhibited high prevalence of S108T and A16V, associated with resistance to cycloguanil, a drug never used in the area. One mosquito mid-gut infection carried the I164L mutant, while another bore a novel I164R variant hitherto undescribed for this locus. Currently considered absent from African *P. falciparum* infections, both S108T and A16V, were initially not found in humans and only subsequently detected in microscopy-negative (submicroscopic) infections. Only wild type I164 was found in human malaria samples. It was concluded that the composition of detectable *P. falciparum* antifolate resistance alleles differs in human and mosquito hosts, presumably reflecting drug and (or) immune selection bias. During epidemiological tracking of drug resistance mutants, it is more representative to sample both human and vector infections.

## 1098

### DEVELOPMENT OF AN ALLELIC DISCRIMINATION ASSAY FOR GENOTYPING SINGLE-NUCLEOTIDE POLYMORPHISMS ASSOCIATED WITH *PLASMODIUM FALCIPARUM* DRUG RESISTANCE

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Global Malaria control programs are being threatened by spread and emergence of old and new drug resistance parasites. To monitor drug resistance, assessment is performed by *in vivo* drug efficacy studies, *in vitro* testing of patient isolates and detection of molecular markers associated with drug resistance. However, *in vivo* drug efficacy studies and *in vitro* testing of patient isolates pose many challenges for a widespread use in resource constraint environment. The use of molecular markers as a tool for assessing *P. falciparum* drug resistance offers an attractive alternative for wide spread profiling of drug resistance that would benefit malaria control. We have developed an Allelic Discrimination TaqMan Assay for genotyping SNPs associated with *P. falciparum* drug resistance performed on an Applied Biosystems PCR platform. This method differs from conventional SNP detection methods in that it can perform parallel analysis of multiple SNPs on an epidemiological scale, it is rapid, inexpensive, sensitive, field deployable and compatible with the current systems found in most laboratories located in malaria endemic areas. To validate our platform, we selected 21 SNPs that are associated with resistance to antimalarials and tested them in nine *P. falciparum* strains that differ in their genetic profile. Of the 189 SNPs tested, 159 have been previously characterized. We obtained three SNPs which were in disagreement with the published information. We confirmed our data was correct by sequencing. Our data show that this assay is capable of discriminating SNP profile of mixed infection and it is highly sensitive. We tested the performance of the assay in the field by analyzing clinical trial samples. Of a total of 735 SNPs evaluated, the assay successfully detected 100% of all the SNPs assayed. We found polymorphism in 14 of the 16 patient samples tested. In conclusion, our assay is robust, reliable, and amenable to throughput.

## 1099

### MALARIA DRUG SENSITIVITY TESTING USING QUANTITATIVE SPECTROSCOPIC ANALYSIS

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Schizont maturation assays have long been a cornerstone in basic malariology to assess parasite growth rates to determine drug susceptibility. With malaria drug resistance increasing in prevalence and severity, new technologies are needed to aid and improve the accuracy and clinical relevance of laboratory or field testing for malaria drug resistance. Here we present a method based on simple and reagentless spectroscopic analysis that provides valuable quantitative information on the morphological and biochemical character of the cells and microorganisms. We used the method to investigate W2 strain of *Plasmodium falciparum* treated with dihydroartemisinin and mefloquine. The size, internal structure, nucleotide and hemozoin composition of the parasites as well as morphology (size and shape) and haemoglobin composition of the infected erythrocytes were determined. Reduction in the sizes of the parasites and their structural organelles was observed after dihydroartemisinin treatment of the ring stage cultures. The nucleotide and hemozoin composition of the treated parasites and haemoglobin composition of the host erythrocytes determined from spectroscopic analysis changed negligibly following the treatment. Although mefloquine treated parasites were growing to the same size as those from parallel non-treated cultures, they lacked hemozoin and had decreased internal structure (organelles). Lesser deformation of the cell shape and no haemoglobin depletion were detected for the infected erythrocytes of mefloquine treated cultures. These results indicate sensitivity of the method for recognition of the effects of antimalarial treatment on the structure and composition of the parasites from the spectroscopic monitoring of the infected erythrocytes. These initial findings in one parasite clone suggest that the spectroscopic analysis can have significant potential for research and clinical applications such as evaluating patient specimens for drug action, drug effects or for therapeutic monitoring.

## 1100

### *IN VIVO* SELECTION OF *PLASMODIUM FALCIPARUM* PFCRT K76 AND PFMDR1 N86 ALLELES BY ARTEMETHER-LUMEFANTRINE IN MALI

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Artemether-lumefantrine (AL) is a highly effective artemisinin combination therapy (ACT) that was adopted in Mali in 2005 as first-line therapy against uncomplicated *Plasmodium falciparum* malaria. This study, designed to measure parasite genetic markers associated with and selected by AL treatment failure, was conducted within the framework of a classical WHO drug-efficacy study. A 28-day follow-up efficacy trial of AL was conducted with 337 total participants (children aged >6 mos and adults) suffering from uncomplicated *falciparum* malaria in 4 different Malian villages -- Faladié (n=88), Kollé (n=77), Bandiagara (n=100), and Pongonon (n=72) -- during the 2009 malaria transmission season. Clinical outcomes in 326 patients (96.7%) were analyzed and the 28-day uncorrected adequate clinical and parasitological response (ACPR) rate was 73.9% -- Faladié (68.2%), Kollé (61.8%), Bandiagara (72.4%), and Pongonon (91.3%). Total PCR-corrected 28-day ACPR was 97.5%. Reinfections and recrudescences were then grouped as recurrent infections and analyzed together by PCR-restriction fragment length polymorphism (RFLP) to identify candidate markers for AL tolerance in the chloroquine resistance transporter gene *(pfcrt)* and the multi-drug resistance gene 1 *(pfmdr1). Pfcrt* T76 population prevalence decreased from 49.3% at baseline (n=337) to 38.8% in patients with *P. falciparum* recurrence (n=85) and *pfmdr1* Y86 population prevalence decreased from 11.0% at baseline (n=337) to 0% in patients with recurrent infection (n=85; P=0.001). Treatment with AL selected for the *pfcrt* K76-allele (P=0.014) and the *pfmdr1* N86-allele (P=0.0002) among recurrent infections. These findings suggest that the *pfcrt* K76T and *pfmdr1* N86Y mutations are associated with enhanced *P. falciparum* susceptibility to AL. Parasite populations exposed to AL in this study selected toward chloroquine-sensitivity, and reinforce observations in E. Africa of *pfcrt* K76 and *pfmdr1* N86 as markers of AL tolerance.

## 1101

### EFFICACY OF ARTESUNATE-AMODIAQUINE (ASAQ) AND ARTEMETHER-LUMEFANTRINE (AL) FIXED DOSE COMBINATIONS (FDCS) FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* (PF) MALARIA IN NIMBA COUNTY, LIBERIA

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Monitoring therapeutic efficacy of recommended artemisinin-based combination therapies (ACTs) for treatment of uncomplicated Pf malaria is essential. Our objective was to assess the efficacy of ASAQ FDC in Liberia, where Pf malaria is largely perennial, and efficacy data scarce. The national antimalarial drug policy was AS + AQ since 2004, and was changed to ASAQ FDC in December 2009. An open-label, randomized controlled non-inferiority trial compared the genotype-corrected Day42 cure rates of ASAQ FDC (ASAQ Winthrop®) to AL (Coartem®) in children <5 years (6% non-inferiority margin; one-sided $\alpha$ 5%, power 80%). Day7 desethyl-amodiaquine and lumefantrine concentrations were measured. Three-hundred children age 6-59 months with uncomplicated Pf malaria, were randomized to 3 days of observed ASAQ (once a day) or AL (twice a day) prescribed by weight. AL was given with fatty food. Parasitaemia, clinical or laboratory adverse events (AEs) were recorded at weekly visits. Day42 genotyping-corrected cure rates were 97.3% (ASAQ; 95%CI: 91.6-99.1) and 94.2 % (AL; 88.1-97.2) (mITT, Kaplan-Meier analysis), and ASAQ was non-inferior to AL (cure rate difference -3.1%, upper limit 95%CI 1.2%). Similar findings were obtained with PP analyses. Day3 parasite clearance rates were 100% (ASAQ) and 99.3% (AL), respectively. Day42 re-infection rates were 43% (ASAQ) and 30 % (AL). Among most frequently reported AEs were fatigue (ASAQ: 28.9%; AL: 13.3%), anemia (ASAQ: 22.8%; AL: 15.3%), cough (ASAQ: 18.8 %; AL: 14.0%), increased liver-enzymes (ASAQ: 12.1%; AL: 16%), and vomiting (ASAQ: 10.7%; AL: 6.7%). The majority of AEs were mild to moderate. Three serious AEs occurred (ASAQ: severe malaria, pneumonia; AL: repeated vomiting of study drug). Both ASAQ and AL were highly efficacious in the < 5year population in Nimba County, Liberia. Re-infection rates were high in both arms in this highly endemic setting. Both FDCs were safe and overall well tolerated. These findings describe for the first time the performance of ACTs in Liberia.

## 1102

### DEVELOPMENT OF NEUTRAL SINGLE NUCLEOTIDE POLYMORPHISM MARKERS TO STUDY THE *IN VIVO* DYNAMICS OF *PLASMODIUM VIVAX* INFECTION

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*Plasmodium vivax* anti-malarial resistance is currently assessed with *in vivo* studies. In these studies, blood samples are taken at weekly intervals after treatment to determine the persistence of the erythrocytic stage. If parasites are found seven days after treatment together with suppressive levels of chloroquine in blood, the parasite is assumed to be resistant. However, *P. vivax* can reappear after treatment through relapses from hypnozoite reactivation, recrudescence of surviving erythrocytic stages or re-infection, making it difficult to determine if the parasite observed after treatment is the one that started the infection. Genotyping the parasites at each time point is the best way to verify parasite identity and exclude new infections during the in vivo study. Variable regions of the Merozoite Surface Protein genes (msp) have been used for genotyping. However, these variable regions are under natural selection and mutations may be selected during the infection process, leading to genotype variation. For this reason, a method to detect neutral single nucleotide polymorphisms (SNP) was designed. Using bioinformatics tools, neutral SNPs of genes located in different chromosomes of P. vivax were identified: msp-1, msp-3 alpha, β-tubulin and cell division cycle-2. An allele-specific hemi-nested PCR assay was optimized to detect the identified SNPs in blood spots preserved in filter paper. Genotypes combining these four genes are presented for well characterized strains from different geographic regions. The use of these P. vivax genotype profiles is proposed as a new tool to study the in vivo dynamics of infection during drug trials.

## 1103

### AMAZON MALARIA INITIATIVE: PROVIDING STRATEGIC SUPPORT TO PREVENT AND ASSIST IN MALARIA CONTROL IN THE AMAZON REGION

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More than 90% of malaria cases in the Americas occur in the Amazon Region. From 1995 to 2000, a significant rise in malaria cases and the emergence and spread of resistance to drugs and insecticides made malaria control a challenge. In 2001, the U.S. Agency for International Development launched the Amazon Malaria Initiative (AMI), establishing a health partnership among six international technical partners and seven countries (Bolivia, Brazil, Colombia, Ecuador, Guyana, Peru and Suriname). Based on capabilities assessments by National Malaria Control Programs (NMCPs), joint work plans for the provision of technical support have been implemented to build sustainable and integrated solutions at local, national and regional levels, through north-south and south-south collaboration. AMI results support that a comprehensive, strategic approach is necessary to strengthen health systems and make a difference in the fight against malaria. AMI's major achievements are the establishment of NMCP's network; the use of evidence-based public health practices by the NMCPs; the improvement of country capacities to monitor resistance to antimalarials and insecticides using *in vivo*, *in vitro* and molecular biology tools; the improvement of the quality control and quality assurance of medicines and insecticides; the transition to Artemisinin based combination therapy following World Health Organization recommendations. Current challenges include sustainability of NMCPs and competing priorities in an epidemiological context.

## 1104

### DEVELOPING AND APPLYING GEOSPATIAL TOOLS FOR MALARIA PREVENTION: AN INTERNATIONAL PARTNERSHIP

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Malaria is a major public health problem in Ethiopia, where outbreaks in highland regions can be affected by climatic variability, land use change, and movements of human populations. Applications of geospatial technologies, including geographic information systems (GIS) and satellite remote sensing, have potential for forecasting the spatial and temporal patterns of malaria risk. Multidisciplinary partnerships can foster the implementation of these tools by linking scientists who have knowledge of geospatial data and techniques with public health practitioners who have a detailed understanding of local needs. We have developed such a partnership involving the Geographic Information Science Center of Excellence (GIScCE) at South Dakota State University, and the Anti-Malaria Association (AMA), a non-governmental organization located in Addis Ababa, Ethiopia. In this partnership, the role of the GIScCE is to

develop models for ecological forecasting of malaria risk using satellite remote sensing, and the role of the AMA is to facilitate data collection, model validation, and implementation of the resulting products. Preliminary results have documented relationships between satellite-derived environmental metrics and malaria incidence in the Ethiopian highlands, confirming the feasibility of environmental risk mapping and forecasting. We have also developed other GIS data products related to land use, health facility accessibility, transportation, and population characteristics that may be useful for enhancan be used to enhance malaria prevention efforts. A key technical challenge in Ethiopia has been implementing internet-based mapping technologies in an environment of low connectivity and low bandwidth. Therefore, another important aspect of the partnership is developing effective, low-cost, and easy-to-use methods for providing public health practitioners with access to digital map products. These approaches include low-bandwidth web applications and standalone applications that do not require internet connectivity. The lessons learned and the tools developed through the ongoing collaboration between GIScCE and AMA can help to inform and enhance other global health partnership efforts.

## 1105

### GENETIC DIVERSITY OF POLYMORPHIC VACCINE CANDIDATE ANTIGENS (*AMA-1*, MSP-3 AND *EBA-175*) IN *PLASMODIUM FALCIPARUM* ISOLATES FROM WESTERN AND CENTRAL AFRICA

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This study was designed to assess the pattern of diversity in three polymorphic genes (*eba-175, msp-3* and *ama-1*) from five countries spanning Central and West Africa. Blood samples were collected from approximately 600 *P. falciparum* infected subjects living in Cameroon, Congo Burkina Faso, Ghana and Senegal. Samples were screened for *eba-175* F- and C- alleles; *msp-3_* K1 and 3D7 alleles; and *ama-1* K1, 3D7 and HB3 alleles using PCR-Digest and nested or semi-nested PCR.. Genetic diversity as measured by mean heterozygosity (*He*) did not differ among genes or countries. However, for some genes, the frequency of alleles did differ among geographic regions. For example, while the eba-175 F-allele predominated in Congo, Cameroon, and Burkina Faso, we found no difference in the frequency of the F- and C- alleles from Ghana and Senegal. Likewise, the frequency of the *ama-1*_3D7 allele was lower in Central African compared to West African countries. Nei's Genetic Distance values for *eba-175* and *msp-3* were close to zero, confirming gene flow while some little high values for *ama-1* (between 0.12 and 0.18) were observed suggesting little or moderate *ama-1* gene flow. The mantel test values, as well as for *eba-175*, msp-3 were close to 0, suggesting the absence or a little of relationship between geographic and genetic distance for each of the maker except for *ama-1* between southern Ghana and Congo (value=0.124), Southern Ghana and Burkina (value=0.118), and Southern and northern Ghana (value=0.100) where mantel test values suggested a little or moderate relationship between geographic and genetic distance.

## 1106

### *IN VITRO* ERYTHROPOIETIC CELL CULTURE FOR STUDIES OF *PLASMODIUM* PARASITES

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Human erythropoietic cells, including reticulocytes, can be produced *in vitro* from hematopoietic stem cells (HSCs) using combinations of cytokines and co-culture with a mouse stromal cell line (MS5). Reticulocytes produced from HSCs have potential for *in vitro* culture of *Plasmodium vivax*. Erythropoietic cells produced from HSCs also have potential for studies of interactions between proteins of *Plasmodium* parasites and their hosts, since erythrocyte proteins can be modified genetically in HSC culture, which is not practical with donor blood. Here, we report use of erythropoietic cells from HSCs for infection of cryopreserved *P. vivax* parasite and for the study of *P. falciparum* host cell receptor. *P. vivax* parasites from infected *Aotus* monkeys were recovered from frozen stock and cultured overnight. Mature parasites were then enriched with centrifugation on 60 % Percoll and mixed with purified reticulocytes from HSCs. After 48 hr culture, newly infected *P. vivax* parasites were observed. Fewer infected RBC were detected after 72 hour incubation. This result suggests that cryopreserved *P. vivax* parasite can invade reticulocytes from HSCs, but culture optimization is required for maturation of infected parasites. To develop a system for study of potential *P. falciparum* erythrocyte surface receptors, HSCs were transfected with siRNA to reduce specific erythrocyte protein levels in maturing RBC. In a pilot study, transfected siRNA specific for Glycophorin C (GPC) resulted in marked reduction of expressed GPC mRNA and protein. Erythrocytes from the transfected HSCs were infected with purified *P. falciparum* parasites. No significant reduction in invasion events was observed with GPC-silenced erythrocytes. Nonetheless, the results suggest that *in vitro* maturation of erythropoietic cells will be useful to study host-blood stage parasite interaction of *Plasmodium* parasites.

## 1107

### A STRUCTURAL AND FUNCTIONAL ANALYSIS OF TWO NOVEL *PLASMODIUM FALCIPARUM* PROTEINS INVOLVED IN THE ERYTHROCYTE INVASION PROCESS

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The clinical symptoms and pathology associated with malaria occur during the asexual erythrocytic phase of the *Plasmodium* life cycle. Central to the severe debilitating effects imposed on the host is the ability of the extracellular merozoites to invade human erythrocytes. Invasion is a tightly controlled process involving specific receptor-ligand interactions between host and parasite molecules. Human erythrocytes are highly polymorphic with respect to the expression of surface molecules. *P. falciparum* has successfully adapted to such diversity by utilizing a number of alternative pathways for invasion that involve distinct erythrocyte receptors. A principal determinant of host cell specificity is the irreversible commitment of the merozoite to the selected host cell by the formation of a junction between merozoite and erythrocyte. The Duffy-binding-like (DBL) family of erythrocyte binding proteins (EBPs), are key parasite ligands that interact with host receptors during invasion. The DBL domain(s) of these proteins are responsible for binding to the receptor molecules. The determination of the *Plasmodium falciparum* genome has enabled other potential paralogues of this family to be identified. Pf10_0348 and Pf10_0355 are two related proteins that contain predicted DBL domains within their structures but unlike other DBL-EBP members also possess structural characteristics found uniquely in the MSP3-like family, making them hybrid molecules. We have utilized both parasite-derived and recombinant proteins to characterize some of the biochemical properties and the

function of these unique molecules. We will also discuss the structure of the DBL domain of Pf10_0355 which we have recently determined to a resolution of 2.8A.

## 1108

### MEASURING GENETIC COMPLEXITY OF *PLASMODIUM VIVAX* INFECTIONS BY A HETERODUPLEX TRACKING ASSAY

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High rates of polyclonal *Plasmodium falciparum* infections in hyperendemic settings are well-characterized, and the ability to measure this genetic complexity affects classification of clinical outcomes in drug efficacy trials. In the same fashion, *P. vivax* infections often contain multiple genetically unique variants even in areas of low transmission intensity such as Thailand. We applied a genotyping strategy that has been successful in measuring genetic complexity of *P. falciparum* infections - the heteroduplex tracking assay (HTA) - to study the complexity of *P. vivax* infections from northwest Thailand. A radiolabeled probe targeting the *P. vivax* merozoite surface protein-1 (PvMSP1) was developed and validated. When annealed with PCR amplified PvMSP1 from different Thai *P. vivax* infections, the HTA detected as many as six variants within a single infection. Comparison of this assay to the more traditional method of RFLP analysis of 40 Thai isolates showed the HTA to consistently reveal more genetic complexity and the ability to uncover minority variants. We plan to use this method to assess the genotypes of relapsing *P. vivax* parasites. This may shed light on the genetic complexity of parasites arising from activation of hypnozoites vs. inoculated parasites in a newly acquired primary infection.

## 1109

### ASSESSMENT OF *PLASMODIUM FALCIPARUM MSP1, MSP2* AND *GLURP* ALLELE DIVERSITY AND FREQUENCY IN SUB-SAHARAN AFRICA

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Molecular genotyping of highly diverse *Plasmodium falciparum msp1*, *msp2* and *glurp* loci is recommended to distinguish recrudescence from new infections in efficacy studies and clinical trials. However, the parasite's genetic profile in many areas has not been systematically documented. Low heterozygosity would result in over-estimation of recrudescence and consequently unnecessary treatment policy changes. Here we present *msp1 msp2* and *glurp* genetic diversity and allele frequency in areas with different transmission intensities namely Malawi, Tanzania, Uganda, Burkina Faso and Sao Tomé. A total 780 baseline (Day 0) blood samples from children under 7 years, from ACT clinical trials done between 1996 and 2000 were genotyped. DNA was extracted; allelic frequency was investigated by PCR followed by genescan for *msp2* and fragment sizing by a digitalized gel imager for *msp1* and *glurp.* The obtained genotypes were further grouped into 5bp, 3bp and 20bp "bins" for *msp1*, *2 and glurp* respectively. Out of 780 DNA samples 599 (76.8%) were successfully amplified at *msp1*, 679 (87%) at *msp2* and 575 (73.7%) at *glurp* loci. The *msp2* gene showed a slightly higher average MOI (2.24), followed by *msp1* (1.48) and lastly *glurp* (1.4). A total of 67 *msp1* genotypes [30 MAD20, 1 Ro33 and 35 K1-types] were recorded. Out of 116 *msp2* genotypes, 83 and 33 represented the 3D7 and FC27 allelic families, respectively. Overall, 31 *glurp* genotypes were detected. All 5 sites recorded very high HE values (0.95 - 0.99). HE was highest in *msp2* locus in Tanzania (HE=0.99), and lowest for glurp in Sao Tomé and Uganda (HE=0.95) (P<0.0001 In conclusion, *P. falciparum msp1, msp2* and *glurp* markers are highly polymorphic and have low allelic frequencies in Sub-Saharan Africa, hence useful in distinguishing *P. falciparum* populations in respective study sites. With the expanding access to ACTs and current changes in malaria epidemiology, allele frequency/genetic diversity should be monitored regularly to ensure reliability of adjusted treatment outcome.

## 1110

### THE *PLASMODIUM VIVAX* TROPHOZOITE PROTEOME: A COMPARISON WITH GENOME AND TRANSCRIPTOME DATA PROVIDES VALUABLE INSIGHTS

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*Plasmodium vivax* causes between 130 to 390 million cases of illness annually. Like other *Plasmodium* species, *P. vivax* antigenically and structurally alters its host red blood cell (RBC) in order to survive. In this study, we constructed the *P. vivax* trophozoite stage proteome using a global proteomic approach; liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is a highly sensitive method by which we identified the peptides extracted from solubilized *P. vivax* Salvador I strain trophozoite infected RBCs, acquired from blood-stage infections in *Saimiri boliviensis* monkeys. We utilized the *P. vivax* genome database to annotate our data and determine the number of known transcripts that are translated into proteins at the trophozoite stage. We report the identification of 688 proteins, which include 191 hypothetical proteins, members of known and recently identified multigene families such as Vir proteins, *Plasmodium* helical interspersed subtelomeric (PHIST) proteins, Pv-fam-a, Pv-fam-d, Pv-fam-e and Pv-fam-h proteins. We analyzed the identified proteins for the presence of export characteristics such as signal peptides, *Plasmodium* export element (PEXEL), and transmembrane motifs. The identification of hypothetical proteins with no known homologs in other species may represent a class of *P. vivax* proteins that could be further characterized as targets for species-specific drug and vaccine development, and also investigated for their contribution to this species' unique characteristics. We compared our *P. vivax* proteome data to published genome and transcriptome data to gain insights into how this species regulates transcription and translation at the trophozoite stage to ensure its survival within the human host. Comparisons with evolutionarily closely related species such as *P. cynomolgi* and *P. knowlesi*, and *P. falciparum*, are also revealing the identity of shared and unique orthologs and enhancing our understanding of expressed proteins and their biological functions.

## 1111

### *PLASMODIUM KNOWLESI* SICAVAR GENE EXPRESSION IN SICA[+] AND SICA[-] CLONES

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The SICAvar variant antigen gene family of *Plasmodium knowlesi* allows for chronic infections in its non-human primate host by the expression of antigenically distinct SICA proteins on the surface of infected erythrocytes, which switch phenotype *in vivo* in the face of immunological challenge. In the rhesus monkey model system, many transcripts representing a host of SICAvar genes can be detected in ring and trophozoite stage infections, however, most are not detected as full length transcripts and they do not go on to be translated into protein and targeted to the

RBC membrane. In this system, the means by which these transcripts are generated and regulated is unknown. Here we show the proteomic and transcriptional profiles of 3 SICA[+] isogenic clones, as well as 2 SICA[-] clones derived from serial subpassages in splenectomized rhesus macaques. Using immunoprecipitation and LC-MS/MS, we identified the major SICA proteins in each of the SICA[+] clones, and confirmed the lack of expressed SICA antigens in the SICA[-] clones. In each SICA[+] clone, there are 2-3 major SICA antigens in terms of abundance, and several others made at lower levels. We also characterized the genes that encode these proteins by quantitative RT-PCR and northern blot, and show that the relative abundance of transcripts matches the proteomic profiles in these clones. By IFA and flow cytometry we attempt to determine whether individual infected RBCs possess multiple SICA antigens. How our findings relate to the orthologous PfEMP1 (var gene) family will be discussed.

## 1112

### ELEVEN *PLASMODIUM VIVAX* MEROZOITE SURFACE PROTEIN-3 (PVMSP3) PROTEINS ARE EXPRESSED AT DIFFERENT LEVELS DURING THE ERYTHROCYTIC SCHIZOGONY

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Members of the *Plasmodium vivax* merozoite surface protein-3 (PvMSP3) are considered potential vaccine candidates because these abundant merozoite surface antigens are exposed to the host's humoral immune system when merozoites are released from the infected erythrocytes. Three members, PvMSP3-α (PvMSP3.10), PvMSP3-β (PvMSP3.3), and PvMSP3-γ (PvMSP3.1) of this gene family have been identified and characterized previously. Here we report the expression profile of the transcripts and proteins of the PvMSP3 family comprising 11 gene members. The hallmark of the PvMSP3 family of proteins is the secondary structure formed by an alanine-rich, central domain (coiled-coil motif) containing variable heptad repeated sequences. These coiled-coil structures span the majority of the coding region. Importantly, the putative MSP3 proteins in *P. vivax*, *P. falciparum*, *P. knowlesi* and *P. cynomolgi* share a conserved Asp-Leu-Arg-Asp (Gly/Ala) motif located 8-17 amino acids downstream from the putative signal peptide cleavage site. We confirmed that all *Pvmsp3* gene transcripts are detected in full-length during the schizont stage and that only two *Pvmsp*3 transcripts change their level of expression dramatically between stages. With exception of PvMSP3.11, all other ten PvMSP3 proteins are expressed during the schizont stage at different levels as determined by quantitative immunoblot. Indirect Fluorescent Assays (IFA) showed that ten PvMSP3 proteins were expressed at surface of merozoites and in the parasitophorous vacuole of schizonts as well. In conclusion, we present a detailed analysis of the gene arrangement, transcript profile and protein expression of eleven members of the MSP3 gene family in *P. vivax*.

## 1113

### INVESTIGATING TEMPO AND MODE IN THE EVOLUTION OF HUMAN MALARIAL PARASITES

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G. G. Simpson, one of the founders of modern evolutionary biology, was interested in variations on the rate of evolution (tempo) and the mechanisms driving those changes (mode). Those are still valid concerns whereas new data from African Apes has raised questions about when and how human malarias originated. In this investigation we are focusing on how can species be defined in the absence of morphological/biological data and how can we time the origin of human malarias? While these may appear to some as "academic questions", such studies provide the basis for comparative genomics. In addition, they allow us to understand host-switches as a mechanism driving the evolution of malarial parasites and to explore the time frames at which novel species could emerge in humans. Whereas it may be difficult to agree upon a " molecular golden standard", mitochondrial genomes (mtDNA) provide reproducible results when used to define species, though the inclusion of nuclear loci is always advisable. Such nuclear loci could be chosen by testing whether their rates of evolution allow for distinction among closely related *Plasmodium* species (e.g. those found in rodents). The use of mtDNA and nuclear loci from parasites found in African apes indicated that two new species closely related to *P. falciparum*, *P. billbrayi* and *P. billcollinsi*, are good phylo-species. However, the dynamics of host-switches among humans and African apes is far more complicated than previously thought and the use of short sequences (e.g. cytochrome b fragments) makes difficult the detection of otherwise well defined phylo-species. In term of timing the origin of human malarias, we explored different methods for estimating the time of origin of *P. falciparum* and *P. vivax* using complete mtDNA. We found that time estimates are seriously affected by the calibration points and violations in the assumptions used by the molecular timing methods. We explore potential calibration points and further discuss the limitations of the mitochondrial genome in estimating the time of origin of malarial parasites.

## 1114

### MOLECULAR BASIS FOR HOST SPECIFICITY IN AVIAN MALARIA

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Malaria is a disease that infects multiple organisms: humans, chimpanzees, reptiles and birds. Malaria is caused by *Plasmodium* blood parasites and has dramatically affected bird populations. The extinction of several avian populations in the Hawaiian Islands was partially due to a newly introduced *Plasmodium* strain that was able to infect multiple bird species (a generalist parasite). Host-specific parasites (specialists) can infect only one bird species. Previous work has shown that specialists can become generalists in host switching events, making them more virulent. The molecular basis of host specificity in avian *Plasmodium* parasites is unknown, but our project aims at identifying genes responsible for host specificity. Previous data has implicated the erythrocyte binding-like (*ebl*)

genes in human and chimpanzee *Plasmodium* strains as potential host-specific determinants. Our studies will focus on identifying the *ebl* genes in avian *Plasmodium* strains infecting African rainforest birds by using PCR to amplify these genes. Currently we have acquired a genetic region for a putative candidate *ebl* gene in *Plamodium gallinaceum,* an avian strain. We further seek to sequence the genes and analyze their DNA and amino acid sequence to determine whether sequence variability correlates with host specificity. We plan to map the distribution of *ebl* gene alleles of African rainforest birds. We expect to identify the *ebl* family of genes in avian malaria and correlate them to host-specificity. The identification of *ebl* genes in avian parasites characterized in this study will allow us to predict potential emerging diseases in avian populations. Predicting potential host-switching events could allow us to slow down the spread of malaria.

## 1115

### COMPARISON OF TWO PCR METHODS FOR THE DETECTION OF *PLASMODIUM SP*. USING DNA OBTAINED FROM MALARIA RAPID DIAGNOSTIC TEST

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Polymerase Chain Reaction (PCR) is a method that has shown a better sensitivity and specificity than microscopy and malaria rapid diagnostic tests (MRDT). It was previously described that the MRDTs can be used as a source of DNA for molecular epidemiology studies. The key for this approach is to have an appropriate DNA extraction method to ensure the quality of DNA to be used in the PCR. The objective of this study was to compare two PCR methods for the malaria diagnosis using DNA extracted from MRDTs using the phenol chloroform method. 187 MRDTs collected in the Department of Loreto, Peru, between February and September 2006 were used as source of DNA. From these MRDTs, 5 were positive to *P. falciparum*, 38 were positive to *P. vivax* and 144 were negative. The DNA was extracted by phenol chloroform method. We used for detection and identification of *Plasmodium* species two different PCR methods using as target the 18S rRNA gene: a Simplex PCR (S-PCR) and Semi Nested Multiplex PCR (SnM-PCR). S-PCR amplifies products of 250 bp for *P. falciparum* and two bands of 220 and 270 bp for *P. vivax*. In SnM-PCR, the first reaction amplifies a band between 783 to 831 bp and in the second reaction a band of 400 bp for *P. falciparum* and a band of 500 pb for *P. vivax*. S-PCR identified 8 *P. falciparum* (S: 57.1%; Sp: 99.4%) and 31 *P. vivax* (S: 63.1%; Sp: 95.3%), while 148 were negative. SnM-PCR identified 5 *P. falciparum* (S: 80% Sp: 99.4%), only 11 *P. vivax*, (S: 23.6%; Sp: 98.6%) and 171 were negative. In conclusion, S-PCR was more sensitive compared with the SnM-PCR because the S-PCR uses smaller DNA target and have more probability to amplify the DNA extracted from MRDTs. Due to the damage during the DNA extraction from the MRDTs, the use of SnM-PCR would not be appropriate because it amplifies longer DNA target.

## 1116

### DISTRIBUTION OF *PLASMODIUM VIVAX* CIRCUMSPOROZOITE PROTEIN SUBTYPES IN DIFFERENT REGIONS OF PERU

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*Plasmodium vivax* is the most prevalent malaria parasite in South America. In Peru most cases are reported from the Amazon basin region. Since this is the most endemic zone of the country, the majority of the genetic diversity studies have been conducted here. However, the rest of the country has largely been neglected for malaria studies. Pvcsp is an important protein on the surface of *P. vivax* sporozoites which is involved in binding to heparin on the surface of hepatocytes and the subsequent invasion of these cells. There are also some published reports that Pvcsp genotype seems to segregate with the ability of the parasite to infect different species of *Anopheles* mosquitoes. Pvcsp has also been used extensively to assess genetic diversity in P vivax populations. There are two subtypes, VK210 and VK247, which differ in the amino acid composition of the central repeat region. In order to identify the *Pvcsp* variants present in different regions of Peru, the *Pvcsp* central repeat region was amplified and sequenced. A high degree of variability was found in 106 samples from Iquitos with 32 alleles of the VK210 subtype found in 98 samples and 3 alleles of the VK247 subtype found in 8 samples. Both subtypes were also found in other zones of the Peruvian jungle, namely, Yurimaguas, southwest of Iquitos, and Madre de Dios, even further to the south. In the central part of the country, from Junin, 10 isolates were genotyped as VK210,with 3 alleles present. Isolates from the North Coast (21 from Piura and 21 from Tumbes) all contained the same allele of the VK247 subtype. The two Pvcsp subtypes are present throughout the jungle of Peru with VK210 more prevalent than VK247. On the North Coast only one allele of the VK247 subtype was detected.

## 1117

### NOVEL MASS SPECTROMETRY APPROACHES FOR THE ANALYSIS OF THE EXPRESSION OF ANTENNAL SOLUBLE OLFACTORY PROTEINS IN *ANOPHELES GAMBIAE*

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Olfactory cues are the most important group of external stimuli affecting mosquito behavior (e.g. mating and partner recognition, search for sugar sources and, in females only, host-seeking and oviposition). Perception of volatile semiochemicals in mosquitoes is mediated by chemosensory neurons segregated within specific olfactory sensilla located mainly on the antennae and maxillary palps. Odourant Binding Proteins (OBPs) and Chemo-Sensory Proteins (CSPs) are soluble proteins responsible of the peri-receptor events leading the detection of odour molecules and to the activation of odorant receptors. *Anopheles gambiae* genome contains a remarkable number of genes encoding OBPs (57) and a lower number of genes encoding CSPs (7). So far, the expression pattern of these genes has been almost exclusively investigated by genomic approaches (i.e. RT-PCR and microarrays): some OBPs have been shown to be specific of the olfactory organs, while others appear to be more ubiquitous. Moreover, OBPs have been found to be differential expressed between males and females and between females at different physiological stages. Traditional proteomic approaches have been proved difficult on mosquito antennae, due to low concentration of OBPs and CSPs in these tissues and difficulties in obtaining sufficient material for 2-dimensional gels. We here present the results of OBPs and CSP expression analyses in *An. gambiae* antennae carried out by an innovative shotgun proteomic approach, based on the nano HPLC-ESI LTQ Orbitrap analysis of the peptides obtained from the enzymatic digestion of the whole protein extract. The sensibility of this approach is compared to that of a more time-consuming traditional approach based on (i) separation of proteins on a 2-dimensional gel, (ii) excision and enzymatic digestion of gel spots, followed by (iii) micro or nano HPLC separation of peptides and (iv) online analysis of peptides through MS and MS/MS experiments on a ESI LTQ Orbitrap mass spectrometer. The advantages/disadvantages of the two approaches are discussed in order to provide a novel perspective in the analyses of soluble proteins in mosquito olfactory organs. Differences observed between male and female antennae are described.

## 1118

### MOLECULAR REGULATION OF AUTOGENY IN THE ARBOVIRAL VECTOR, *CULEX TARSALIS*

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Autogeny, the ability of a female mosquito to produce eggs without blood feeding, is a alternate reproductive strategy with important implications for vector-borne disease transmission. Very little is known about the molecular mechanisms that regulate autogeny and how they compare with blood meal regulated reproduction (anautogeny). In order to compare these reproductive strategies, we selected for and characterized autogenous and anautogenous populations of an important arboviral vector, *Culex tarsalis*. While autogeny is a genetic trait, its expression in *C. tarsalis* is severely compromised by the availability of adequate larval nutrition, similar to the availability of nutrients following a blood meal in anautogeny. We cloned components of two nutrient-sensitive pathways with demonstrated functions in anautogeny in other mosquito species: *C. tarsalis* Target of Rapamycin (CtTOR), the kinase that functions in the TOR signaling pathway, and the *C. tarsalis* Insulin Receptor (CtInR), the receptor for the insulin signaling pathway. We investigated tissue specific expression and used RNAi mediated knockdown to examine the effect of these pathways on vitellogenin gene expression and egg development in our selected *C. tarsalis* populations.

## 1119

### MOLECULAR CLONING OF THE SODIUM-METHIONINE SELECTIVE SYMPORTER FROM THE YELLOW FEVER MOSQUITO

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Nutrient Amino acid Transporters (NATs) comprise a recently identified subfamily of the Neurotransmitter Sodium Symporter Family (NSS or SLC6). In vector mosquitoes NAT-SLC6 population includes a large number of paralogous (species-specific) transporters with unknown physiological properties and biological functions. Here we report the molecular cloning, functional heterologous expression, and in situ hybridization of AeNAT5, a new insect NAT from the yellow fever mosquito, *Aedes aegypti* (NCBI accession # ABZ81822). In contrast to the previously characterized an Aedes broad spectrum AAT1 and the *Anopheles* L-tryptophan- and L-phenylalanine- selective transporters, AgNAT6 and AgNAT8 respectively, AeNAT5 selectively absorbs L-methionine (K 0.5 L-Met = 20+/-9 uM; K0.5 Na+ = 46 +/-17 mM; stoichiometry 1:1) and, with ~20 fold reduced transport efficiency, L- cysteine and Homocysteine. It rejects other canonical amino acids and amine neurotransmitters. AeNAT5 transcript is abundant in the larval alimentary canal. However, substantial differences in expressions of AeNAT5 vs. AeAAT1 were found in the anterior and posterior domains of the larval gut. AeNAT5 is also identified in the mosquito brain and a few other tissues. These findings are consistent with our earlier proposal that the NAT populations evolve and act synergistically as a principal molecular mechanism of absorption and redistribution of essential amino acids. The narrow substrate spectra AeNAT5, AgNAT6 and AgNAT8 comprise a mosquito-specific addition to the NAT population, which balances the acquisition of the most underrepresented essential amino acids vs. more redundant nutrients. Hence, the essential NATs may be especially critical for extensive protein synthesis during mosquito development and egg maturation. The essential linage-specific NATs may provide more specific targets for the management of medically and economically important insects.

## 1120

### CHARACTERIZATION OF DOUBLE STRANDED RNA BINDING PROTEINS INVOLVED IN THE RNA INTERFERENCE PATHWAY OF THE DENGUE MOSQUITO, *AEDES AEGYPTI*

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*Aedes aegypti* mosquitoes are the vectors of several arboviruses of global health significance, including dengue viruses and yellow fever virus. The RNA interference (RNAi) pathway is an important defense mechanism used by invertebrate organisms to protect against viral infection, and we have shown previously that RNAi plays a direct role in vector competence. While the structure and function of many genes involved in the *Drosophila* RNAi pathway have been characterized, the corresponding mosquito orthologs have only been peripherally described or remain unknown. We have characterized the gene structure of *Ae. aegypti* double stranded RNA binding proteins predicted to be important to the RNAi pathway. Two genes, *r2d2* and *r3d1* are orthologs of *Drosophila* genes known to have important roles in the RNAi initiator complex. A third member of the same family, which we refer to as *extra loquacious* (*exloqs*), appears to have no known orthologs outside of the *Aedes* genus. Our characterization of these genes has revealed new exons and alternative splice variants for both *r3d1* and *exloqs*. In addition, we examined single-nucleotide polymorphisms, as well as differential expression of all three genes for specific tissues and developmental stages. This work increases the accuracy of the annotations for all three genes, and provides valuable insight for future mechanistic and biochemical studies involving these gene products and their roles in RNAi.

## 1121

### VALIDATION OF NOVEL PROMOTER SEQUENCES DERIVED FROM TWO ENDOGENOUS UBIQUITIN GENES IN TRANSGENIC *AEDES AEGYPTI*

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To date, only a limited number of promoter sequences have been described to drive transgene expression in the disease vector *Aedes aegypti*. We sought to increase this repertoire by characterizing the ability of upstream sequences derived from the *Ae. aegypti* UbL40 and polyubiquitin genes to drive the expression of marker proteins. Both genomic fragments were able drive robust expression of luciferase in cultured mosquito cells. Following Mos1-transformation, the UbL40 promoter drove strong expression of a fluorescent marker in early larvae and in ovaries, while the polyubiquitin promoter drove robust EGFP expression in all stages of development, including constitutive expression throughout the midgut. In addition, both promoters drove robust expression of luciferase within 12 hours after injection into newly laid embryos. These promoter elements will thus be useful for the expression of anti-pathogen effector genes in genetics-based control strategies; hairpin RNAs in gene knockdown experiments; and as drivers of transposase/recombinase expression in new helper systems.

## 1122

### INCREASED INSULIN SIGNALING IN TWO KEY REPRODUCTIVE TISSUES, THE FAT BODY AND OVARIES, INCREASES MOSQUITO FECUNDITY

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Mosquito reproduction is regulated by a complex series of hormonal cues. In the ovaries and fat body the insulin/insulin growth factor 1 signaling (IIS) cascade regulates steroidogenesis and vitellogenesis respectively.

Phosphotase and tensin homolog (PTEN) is a direct antagonist of IIS. Knockdown of AaegPTEN or its specific splice variant AaegPTEN6 by RNAi resulted in a 15-63% increase in egg production and had no effect on the egg viability during the first reproductive cycle. Knockdown of a second splice variant, AaegPTEN3, had no effect on reproduction. However, RNAi has limitations. The dsRNA injections resulted in knockdown of AaegPTEN in both fat body and ovaries making it impossible to elucidate whether the ovaries fat body, or both tissues are responsible for the increased egg production. In addition, the transient nature of RNAi knockdown prevents us from determining whether increased reproduction occurs throughout the life of the mosquito or whether there is a life history change to earlier reproduction. To address these questions we are generating transgenic mosquitoes with either increased or decreased IIS in the fat body and ovary. We have successfully engineered transgenic lines using the vitellogenin promoter linked to an insulin signaling inhibitor, PTEN, or to its activator, Akt. We characterized how changes to IIS specifically in the fat body effects egg production during multiple reproductive cycles and the impact is has on mosquito lifespan. We have also identified a putative ovary specific promoter with expression in the ovarian follicle cells where the IIS cascade regulates steroidogenesis. We have linked this promoter to an activator of the IIS cascade and are generating and characterizing transgenic lines with modified IIS in the ovaries.

## 1123

### TEMPORAL SILENCING OF TWO *CULEX PIPIENS QUINQUEFASCIATUS* (DIPTERA: CULICIDAE) MOSQUITO GENES: METHOD FOR SILENCING POTENTIAL IMMUNE RESPONSE GENES

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A mosquito's ability to transmit a virus is influenced in part by how active its immune response is to the invading virus. Components of the mosquito Toll and immune deficiency pathways are known to counter foreign pathogens, including viruses. Preliminary studies on gene expression responses in midgut tissues of *Culex pipiens quinquefasciatus* infected with West Nile virus (WNV) revealed two genes, one encoding a Toll-like protein and the other a gram-negative bacteria binding protein, whose expression was altered after infection. Changes in expression after viral infection could indicate that these genes are members of the immune response pathway. Injection of double-stranded (ds) RNA representing the Toll-like gene, CQ G12A2, into *Cx. p. quinquefasciatus* showed decreased expression in midgut tissue beginning 6 days post-injection (dpi) through 9 dpi. Blood feeding the dsRNA-injected mosquitoes 4 days after injections shifted the expression knockdown in midguts to 8 dpi. Injection of dsRNA representing the gram-negative bacteria binding protein gene from *Cx. p. quinquefasciatus*, CQ G1A1, knocked out the expression in midgut tissue 1 dpi through 9 dpi. When these injected mosquitoes were blood fed 4 days following injection, gene expression levels did not decrease until 7 dpi. Here we present results that indicate the importance of tracking the time required for change in expression for each RNA target. With this information we can pinpoint the time of maximum knockdown to study the involvement of these genes in mosquito-WNV interactions. We also discuss the role of blood ingestion in delaying gene expression knockdown; this issue should be considered in studies that use RNAi technology to assign a gene a function in vector competence.

## 1124

### SPATIAL AND TEMPORAL PATTERNS OF GENE EXPRESSION IN SALIVARY GLANDS OF *AEDES AEGYPTI*

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Transgenic mosquitoes designed to impact vector competence have been proposed as tools for the control of dengue viral transmission. In addition to the development of anti-dengue effector molecules that block viral replication and dissemination, promoters are needed that target effector gene expression to key mosquito tissues where the viruses and host interact. It has been shown in transgenic mosquitoes that expression of anti-dengue effector molecules in the distal-lateral lobes of *Aedes aegypti* salivary glands reduces prevalence and mean intensities of infection of the virus. We anticipate greater efficacy of viral suppression if we target the effector genes to all lobes of the salivary glands. We report here the hybridization in situ patterns of 19 genes expressed in the salivary glands of adult *Ae. aegypti* females. Distinct spatial expression patterns were identified. Eight genes are expressed exclusively in the proximal-lateral lobes, five genes within the distal-lateral lobes, and two genes in the medial lobe. Four genes are expressed in the distal-lateral and medial lobes. Quantitative real-time RT-PCR was used to measure relative levels of gene expression following blood feeding for seven genes that represent the four major classes of spatial expression patterns. All analyzed genes are expressed constitutively. It is possible that continuous expression of anti-viral effector molecules throughout the salivary glands will completely disrupt dengue transmission. Based on our data, a minimum of two promoters is necessary to drive the expression of one or more anti-dengue genes in all cells of the female salivary gland.

## 1125

### INSULIN SIGNALING IN THE MOSQUITO: UNDERSTANDING AKT PHYSIOLOGY IN THE FAT BODY AND PTEN REGULATION AT THE MOLECULAR LEVEL

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The insulin/insulin growth factor 1 signaling (IIS) cascade regulates aging, reproduction, and innate immunity in a wide range of organisms. To assess the impact of IIS in mosquitoes we examined two critical IIS molecules, the inhibitor Phosphatase and Tensin homolog (PTEN) and the activator Akt. We generated transgenic *Anopheles* stephensi lines overexpressing an active Akt (myr-AsteAkt) regulated by the vitellogenin promoter to enhance IIS activity in the fat body. We assessed mRNA transcript and protein expression levels, and myr-AsteAkt was expressed only in the fat body in a bloodmeal dependant manner as expected. The impact of increased IIS on lifespan and reproduction is examined. This work assesses the link between changes in lifespan, reproduction, innate immunity and IIS manipulation in the fat body. We are also studying the biochemistry of the key IIS inhibitor in the mosquito, PTEN. Regulation of PTEN is critical for its lipid phosphatase activity, membrane association, and subcellular localization. One of the key regulatory mechanisms of PTEN is the phosphorylation of serine and threonine residues leading to decreased enzymatic activity. Using a proteomics approach, we identified several phosphorylated serine and threonine residues on the C-terminus of the mosquito PTEN6. However, the kinases involved and the signaling context of the phosphorylation is unknown. Sequence analysis predicts several phosphorylation sites on *Aedes aegypti* PTEN6. Four sites on the C-terminal regulatory region are predicted as Casein Kinase I (CK1) and Casein Kinase II (CK2) substrate sites. *In vitro* kinase assays were used to determine if recombinant mosquito PTEN acts as a substrate for CK1 and CK2. Understanding how PTEN is regulated at the molecular level will allow us to utilize this IIS inhibitor in unique mosquito control strategies.

## 1126

### DEFECT OF DSRNA PROCESSING IN *AEDES ALBOPICTUS* C6/36 CELLS

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Double-stranded RNA (dsRNA) is the trigger of RNA interference (RNAi)-mediated gene regulation. Dicer protein processes dsRNAs into short interfering RNAs (siRNAs), which are incorporated into the effector RNA induced silencing complex and direct degradation of homologous target mRNAs. In plants and insects, RNAi can acts as an antiviral mechanism through generation of viral specific siRNAs from a replicating virus by Dicer. In this study, we analyzed the RNAi machinery in mosquito C6/36 cells, an *Aedes albopictus* cell line commonly used for propagation of dengue virus and some other flaviviruses. Transfection of long dsRNAs (~500bp) did not result in specific knockdown of cognate reporter genes (GFP and Renilla luciferase) in C6/36 cells, and showed no significant difference between the effects caused by specific target dsRNAs and unrelated dsRNAs. However, expression of the GFP gene can be efficiently inhibited by chemically synthesized GFP siRNA, indicating that there may be defects in dsRNA processing in C6/36 cells. To test this possibility, *in vitro* Dicer assays were performed by using crude cell extracts prepared from C6/36, *Aedes aegypti* Aag2, *Anopheles gambiae* 4a-2s4 and Drosophila melanogaster S2 cells. Consistent with the inefficacy of dsRNAs in the cell-based analysis, production of siRNAs was not detected when radiolabeled long dsRNAs were incubated with the C6/36 extract. In contrast, dsRNAs were efficiently processed into siRNAs in extracts from all other cell lines. This result suggests that in C6/36 cells dsRNA processing in RNAi pathway is defective and such a property may provide an advantage to virus replication.

## 1127

### MACROPHAGE NUCLEAR RECEPTOR SIGNALING MODULATES DEFENSES AND SUSCEPTIBILITY TO MYCOBACTERIUM TUBERCULOSIS

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We are studying host pharmacological effectors, contributing towards Mycobacterium tuberculosis (*Mtb*) pathogenesis or clearance. The project has a "look beyond NF-kB approach" an overrated pharmacological molecule in macrophages, to other orphan/ nuclear receptor that have recently been reported to find expression in immune cells such as macrophages. Preliminary observations suggest that these orphan/ nuclear receptors may contribute towards susceptibility or resistance of natural host. We have studied differential expression of orphan/ nuclear receptor in macrophages. We have confirmed altered expression of these receptors to H37Ra strain. We have confirmed responding receptors by cell based bug survival assays and using avirulent strains such as *BCG*, *M. smegmatis*, *H37Ra*. We have identified that these receptors have pro-*Mtb*/ anti-*Mtb*/ neut-*Mtb* function largely by promoting/ resisting macrophage foam cell formation. Some of these receptors are 'Lipid Sensing', to mean that while some of them have lipid as their ligands, others have lipids as post translational modification. We are studying host-pathogen interaction at level of *Mtb* lipid repertoire to modulate these LSNRs. All the above studies are being verified to virulent strain H37Rv in cell based and animal based assays. Also clinical samples from human volunteers from interesting family history are being attempted to be looked for differential expression of these factors in monocytes/macrophages derived there from.

## 1128

### A PROSPECTIVE COHORT STUDY TO EVALUATE INCIDENCE OF TUBERCULOSIS IN INFANTS, WESTERN KENYA

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Cohort studies which include comprehensive diagnostic methods to provide reliable estimates of TB incidence and other epidemiological parameters in infants are needed to guide the planning of future TB vaccine trials. We set out to determine the incidence of TB, latent TB infection and all cause and TB specific mortality rates in Siaya district, western Kenya. To demonstrate a TB incidence of 0.5%, and make inferences for a phase III trial, ~2900 infants are being enrolled and followed up for a minimum of one year. Through 4-monthly follow up visits and health facility (HF) surveillance, those determined to be TB suspects by history of contact, TB symptoms, hospitalisation history, are admitted to a case verification ward. Specimens are collected for microscopy and culture by induced sputum and gastric aspiration. Chest radiographs, mantoux tests, and HIV testing are performed. Additional morbidity and mortality surveillance is conducted through HF record reviews. From June 2009-February 2010, 1553 BCG vaccinated infants have been enrolled with cumulative follow up of 325 person years. Of 1553 participants, 199(12%) were TB suspects. Of those 3(1%) had a history of contact with a TB case, 34(15%) had TB symptoms, and 139(70%) had protocol defined hospitalisation history. Six incident TB cases(2%) have been identified based on suggestive radiological and clinical features, however, none have been confirmed by bacteriology. Two of these are HIV coinfected, and 4(1%) are eligible for Isoniazid therapy following positive mantoux, negative cultures and symptoms. In conclusion, our preliminary analysis suggests that the majority of the TB suspects and TB cases were generated from the protocol defined hospitalisation history. To intensify case finding in infants in TB vaccine trials, the criteria for TB suspect identification need to be reconsidered, and go beyond contact history and Tb symptoms.

## 1129

### DIFFERENTIAL RELATIONSHIP OF ENDOGENOUSLY ACTIVATED TH1/TH2 CYTOKINE SECRETING CELLS IN PULMONARY TUBERCULOSIS AND HEALTHY COMMUNITY CONTROLS IN A BCG VACCINATED HIGH TB BURDEN COUNTRY

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Pakistan ranks 8$^{th}$ among 22 high TB burden countries despite wide BCG coverage (>90%). Tuberculin skin test positivity (TST+) in healthy community controls is as high as 50% increasing to 80% in recently exposed contacts. T effector cells are activated in the lymphoid compartment, develop homing receptors (CCR7) and transit to infected tissue sites via the blood compartment. We hypothesized that the relationship of Th1 and Th2 activated cells in transit may be different in tuberculosis patients compared to healthy controls. We therefore, analyzed the relationship of Th1 (IFNγ, IL2, TNF α) and Th2 (IL4 and IL6, IL10) cytokine secreting cells in the peripheral blood compartment of tuberculosis patients with active pulmonary disease (PTB=17) compared to healthy controls with latent infection (EC TST+=18). Whole blood (1:10) was cultured in the absence of exogenous stimulus for 2 days and

supernatants were tested for Th1/Th2 cytokine using the Cytometric Bead Array system. All Th1/Th2 cytokines were significantly elevated (Mann Whitney U test $p < 0.01$) in PTB compared to EC TST+. Spearman Rank analysis was carried out to determine the relationship between Th1 and Th2 cytokines. Differential association between Th1 and Th2 cytokines in the two groups was observed with IFNγ Vs IL6 (PTB, rho= -0.023, p>0.1; EC TST+, rho= 0.665, p=0.003) and between IL2 Vs IL4 (PTB, rho= -0.419, p=0.083; EC TST+, rho=0.668, p=0.002). Differential endogenous activation of Th1/Th2 cytokines in pulmonary tuberculosis and healthy TST+ community controls may have important implications with respect to disease pathogenesis and protection.

## 1130

### THE USE OF MOLECULAR TECHNIQUES FOR THE IDENTIFICATION OF *MYCOBACTERIUM BOVIS*

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*Mycobacterium bovis*, member of *M. tuberculosis* complex (MTBC) causes tuberculosis (TB) mainly in cattle but has a broad host range and causes disease similar to that caused by *M. tuberculosis* in humans. Identification of *M. bovis* traditionally has been based on phenotypic characteristics and biochemical properties.Several molecular methods have been developed for the identification of *M. bovis* including DNA sequence variations in the direct repeat region of MTBC complex -spoligotyping or single nucleotide polymorphisms (SNPs) in the *oxyR* gene or be differentiated by large sequence polymorphisms or regions of difference (RD). The objective of this study was to determine a molecular method for the Detection of *M. bovis* from cattle. 17 suspected lesions from positive rectors (cattle) from Comparative tuberculin test were cultivated on LJ medium containing pyruvate. Isolates were identified using biochemical assays and PCR using Insertion sequence IS6110, Allele-specific(oxyR) and Spoligotyping Three(16%) of the isolates gave phenotypic properties were characteristic of (MTBC) while the remaining three were identified as non tuberculosis mycobacteria Insertion sequence of isolates gave 50% identification while with *oxyR* 3(50%) were identified as *M. tuberculosis*. Spoligotyping identified *Mycobacterium tuberculosis Ghana*, *Mycobacterium africanum,*while sequencing of the 16rRNA identified two non tuberculosis bacteria - *Mycobacterium. fla*vescens and *Mycobacterium. moriokaense* which have been known to infect animals example cattle presenting histological form similar to those presented in cattle infected with *Mycobacterium bovis.* As far as can be gathered from literature this is the first time *Mycobacterium tuberculosis* in cattle has been identified in Ghana through molecular typing of appropriate isolates.

## 1131

### CAN JOB TITLES BE PREDICTORS FOR RECENT ONSET LATENT TUBERCULOSIS IN HEALTH CARE WORKERS?

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Latent tuberculosis (LTB) is the stage of *Mycobacterium tuberculosis* that is asymptomatic, dormant and non-contagious. Although health care workers are considered as high risk for LTB, it has been debating if job types are associated with the risk of LTB. In addition, there is limited data of this issue on the recent onset LTB. We determined the association of job types and tuberculin conversion or recent onset latent tuberculosis in healthcare workers in an endemic area of tuberculosis. A case-control study was done at Srinagarind hospital, Thailand. Cases were subjects with tuberculin conversion, while controls were subjects with negative results of tuberculin skin test (TST) in two consecutive years. There were 1,025 subjects completed two consecutive TST during 2001-2009. The incidence rate of tuberculin conversion was 19.8% or 203 subjects. In a multivariate model, the only three significant factors for tuberculin conversion were male gender, having BCG scar, and job types. Only nurses, nurse assistants, and workers were significantly associated with tuberculin conversion with adjusted odds ratio [95% confidence interval] of 2.3 [1.3-4.1], 2.3 [1.3-4.7], and 3.0 [1.8-5.0], respectively. Tuberculosis infection control program should be emphasized in those job types of healthcare workers who are at risk.

## 1132

### COMPARISON OF DIFFERENT MOLECULAR AND CULTURE-BASED STOOL TECHNIQUES IN PULMONARY TUBERCULOSIS DIAGNOSIS

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The diagnosis of pulmonary tuberculosis (TB) is difficult in patients unable to provide sputum. Most sputum is swallowed and we evaluated molecular and culture-based tests for detecting *M. tuberculosis* from swallowed sputum in stool for the diagnosis of pulmonary TB. Stool samples from adults with suspected and proven pulmonary TB, prior to and during treatment were tested. The diagnostic performance of the following techniques was compared: an IS6110 nested polymerase chain reaction (PCR); sputum smear fluorescence microscopy with centrifuge concentration and Auramine staining; the Microscopic-Observation Drug-Susceptibility (MODS) broth culture technique; culture on antibiotic-enriched selective Middlebrook 7H10 thin-layer agar (TLA); and culture on conventional Lowenstein-Jensen (LJ) solid culture medium. Stool was decontaminated with the NALC-NaOH technique as used for sputum. Of 1,086 stool samples, 129 were culture positive. For these samples, the diagnostic sensitivity of MODS was 92%, higher than LJ (81%, P=0.02), PCR (75%, P<0.01), all of which were more sensitive than TLA 59%, P<0.01) and only 40% were microscopy positive. Considering the 934 samples with results for all tests, PCR was positive for 19% and culture 12%: MODS in 9.2%, LJ in 7.3%, TLA in 6.0% and microscopy in 5.3% (all comparisons P<0.01). Contamination caused test failure for 1.8% of MODS tests, 3.4% of TLA and significantly more for LJ cultures (15.2%, P<0.01). 567 of the PCR were performed after two DNA extraction techniques and positivity was significantly more frequent with commercial spin columns (Qiagen), than the in-house Chelex technique (16% vs 12%, P=0.03). In conclusion, PCR of stool specimens has higher sensitivity than culture for the diagnosis of pulmonary TB. Qiagen columns performed better than Chelex extraction. MODS had the highest sensitivity and lowest contamination rates among the three culture techniques.

## 1133

### GENETIC VARIABILITY IN HUMAN METAPNEUMOVIRUS CIRCULATING IN CENTRAL AND SOUTH AMERICA

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The human metapneumovirus (hMPV) is a recently discovered member of the family Paramiyxoviridae responsible for acute respiratory tract infections in young children, elderly patients, and immunocompromised hosts. Based on genomic sequencing and phylogenetic analysis, there are two major hMPV subtypes: A and B. These analyses are based on the sequencing of the N, M, F, G, or L genes and genotype grouping are concordant regardless of which gene is studied. The major differences between the A and B genotypes are nucleotide polymorphisms concentrated mostly on the G and SH proteins. The G gene of hMPV displays significant strain-to-strain variability. In this study we genetically analyzed the circulating hPMV in Central and South America from July 2008 to June 2009 and characterized the strains present in this region and their genetic variability. Samples were collected during an international collaborative febrile surveillance study. All were cultured and analyzed by inmunofluorescence for influenza, hRSV, parainfluenza, enterovirus, adenovirus, herpes simplex virus (1 and 2), and hMPV. Only those hMPV culture-postive samples were confirmed by RT-PCR and sequenced. The primers used were specific to hMPV G and N genes. This study analyzed 32 culture-positive samples. Of these, 50% were male and 50% were female. Nineteen of the samples came from children under 12 years of age, 3 from adolescents age 12-17, 7 from adults age 18-50, and 3 from participants older than 50 years.

Nucleotide comparison of the samples revealed the existence of two major genetic clusters. The phylogenetic analysis for the N gene of samples showed high similarity between the viruses, while the amino acid sequence of G gene clearly showed more diversity in the strains; genotypes A2, B1, and B2 were detected. The partial comparison of amino acid sequences of the G protein of hMPV revealed that all changes were base substitutions, with no deletions or insertions. In conclusion, our results show that two distinct clusters of hMPV circulated in Central and South America during the July 2008- July 2009 period. Similar clusters were reported in Canada in 2002, suggesting that a relative homogeneous population of hMPV is circulating throughout the world. These results differ from the previously described isolated viruses in 2003 in Peru which showed subtype A as the predominant subtype.

## 1134

### CLIMATE AS SEASONAL INFLUENZA PREDICTORS

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Seasonal influenza continues to be a significant public health burden. Despite vaccination and the largely mild cases, influenza causes up to 300,000 deaths each year worldwide. Moreover, influenza virus inherently undergoes rapid mutation that has the potential to bring about pandemic at any time. Hence understanding transmission pattern and capabilities to accurately project influenza cases can contribute to reducing the disease burden, as well as facilitating the preparedness effort. Toward this end, our group has examined the role of climatic and environmental factors in influenza seasonality. We have previously shown the dependencies between climate and influenza incidences in two regions with warm temperature. We now extend our analysis to cities with temperate climate, and subsequently compare the dominating climatic indicators between the regions. Remotely-sensed climatic indicators from NASA satellites - such as Land Surface Temperature (LST), precipitation as a measure for rainfall - as well as meteorological measures from the ground stations are used as covariates in our empirical models. In general, the resulting model can identify the timing of influenza peak reasonably well. We further produced influenza forecasts for the following season using the climate-based model. The resulting one-season-ahead prediction provides baseline cases that can be used to estimate vaccine production. The identification of influenza-associated environmental parameters from this work could also guide further biological studies on the transmission mechanism.

## 1135

### ACUTE RESPIRATORY INFECTION, MAIN CAUSE FOR MORBIDITY FOR CHILDREN 0-5 YEARS OF AGE, IN POST-EARTHQUAKE, HAITI, 2010

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An earthquake measuring 7.0 struck Haiti on January 12th, 2010 at 16:53hours local time. It had devastating effect on the capital Port-au-Prince, the towns of Carrefour, Leogane, Grand Goave, and Jacmel in the South East Department. A total of 3million people were affected by the earthquake of whom 2million are estimated to have been displaced from their homes. Save the Children, a humanitarian organization, that has been operational in Haiti for 21 years responded immediately by establishing mobile medical team and providing emergency medical services in Port-au-Prince, Leogane, and Jacmel. Disease surveillance is one of the key tasks of the medical team to prioritize health action and determine the occurrence of disease outbreaks. From January 31st - April 4th, 21 Mobile Medical team of Save the Children composed of medical doctors, midwife and nurses, had 52,761 consultations in Port-au-Prince and Jacmel, out of which 22% were due to Acute Respiratory Infections (ARIs). ARI accounted for 48.4% of consultations among children 0-5yr of age, and 12.5% among those over the age of 5yrs. The total consultation due to ARIs was four fold higher than the total number of consultations due to diarrhea and suspected malaria among children 0-5yr of age. The World Health Organization (WHO), estimates that prior to the earthquake pneumonia accounted for 20% of mortality among children in Haiti, much higher than diarrhea (16%) and malaria (1%). Crowded leaving conditions, low vaccination coverage, and poor nutritional situation have exacerbated the risk for pneumonia among children in post-earthquake Haiti. Efforts to have a strict case definition for pneumonia in the surveillance system is critical to monitor disease trend, prioritize public health action including advocacy to improve shelter, food, and vaccination among the internally displaced persons in Haiti.

## 1136

### EFFICACY OF A RAPID DIAGNOSTIC ASSAY FOR DETECTING PANDEMIC INFLUENZA A: H1N1 IN A RESPIRATORY SURVEILLANCE COHORT IN PERU

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A population-based active surveillance cohort to estimate the burden of influenza and influenza like illness (ILI) was initiated in 4 geographically distinct regions of Peru in June-July 2009, in the early phase of the pandemic. A rapid diagnostic assay, QuickVue Influenza A+B®, was performed in the field as the first step in diagnosis of influenza among ILI cases. In the context of the pandemic, we evaluated the sensitivity, specificity and usefulness of this test as a tool to aid clinical and public health decisions. Nasal/throat swabs were collected from subjects with ILI (fever ≥38°C with cough and/or sore throat). For this study, in order to evaluate the usefulness of this test during periods of higher virus circulation, we selected samples collected during the peak of the pandemic in Lima (A), Tumbes (B), and Puerto Maldonado (C). The fourth site was excluded because a pandemic peak could not be clearly defined.

Epidemic peak periods were selected considering the highest ILI attack rates per site observed in 2009. Samples were tested by QuickVue A+B® within 24 hours of ILI case detection, stored at -70ºC, transported to the lab and tested by real-time PCR for pH1N1. Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) per site were calculated in order to measure the usefulness of the test. A total of 570 samples were collected during selected epidemiological weeks (EW): EW 25-29 for A (n=186), and EW 37-41 for B (n=248) and C (n=136). The specificity was similar in these sites: 96, 97, and 100% for A, C and B, respectively. However, the sensitivity was variable: 38, 48 and 55% in B, C and A, respectively. Similar results were obtained for PPV: 94, 97, 100, and 94% in C, A and B, respectively. The NPV was 47% in A, 63% in C and 70% in B. In conclusion, the sensitivity of the QuickVue Influenza A+B® appears to be low for the specific pH1N1 genotype. As expected, NPV decreased when prevalence of infection was higher in the population, as in Lima. An NPV as low as 47%, under this context, makes the test of limited use as a screening tool during a pandemic. Therefore, final diagnoses should be confirmed with the accepted gold standard (real-time PCR for pH1N1). However, while this particular rapid diagnostic assay suffers from low sensitivity rates, utilization of such assays allows clinicians to make informed decisions and respond in a more rapid fashion.

## 1137

### *MYCOBACTERIUM LEPRAE* IS SUSCEPTIBLE TO CEM-101

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CEM-101, a new macrolide-ketolide in clinical development, has been found to be a minimum of 4-fold more active than other macrolides, mainly clarithromycin and azithromycin and 2-4 fold more active than telithromycin. It is active against a variety of macrolide-resistant pathogenic strains of *S. aureus*, *S. pyogenes*, and *S. pneumoniae*. The efficacy of CEM-101 against *Mycobacterium leprae*, the causative agent for leprosy, was investigated in the present study. The Thai-53 isolate of *M. leprae*, maintained by serial passages in athymic *nu/nu* mice footpads, was used for all experiments. For axenic testing freshly harvested viable *M. leprae* were incubated in medium along with different concentrations of the drugs (CEM-101, clarithromycin and rifampin) for 7 days at 33oC. At the end of this incubation drug-treated *M. leprae* were subjected to radiorespirometry to assess viability based on oxidation of 14C palmitate and staining with viability dyes to assess the extent of membrane damage. For intracellular testing peritoneal macrophages from Swiss mice were infected with freshly harvested viable *M. leprae* at a MOI of 20:1 for 12 hours. At the end of the infection extracellular bacteria were washed and drugs added at different concentrations and incubated for 3 days at 33oC. At the end of 3 days cells were lysed to obtain the intracellular *M. leprae* for viability testing by radiorespirometry and viability staining. CEM-101 at 0.15 µg/ml was able to significantly ($P$<0.001) reduce the viability of *M. leprae* in both axenic and intracellular cultures when compared to controls. Inhibition by CEM-101 was not statistically different from inhibition obtained with clarithromycin under identical conditions and at the same concentration against the claithromycin-susceptible Thai-53 strain. In conclusion, CEM-101 is effective against *M. leprae* potentially expanding the drugs available to treat leprosy.

## 1138

### CHANGES IN ILI CASE REPORTING PATTERN IN AN ELECTRONIC SURVEILLANCE SYSTEM DUE TO PANDEMIC INFLUENZA A (PH1N1) IN THE PERUVIAN NAVY, 2008-2009

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During 2008-2009, the Department of Health of the Peruvian Navy closely monitored influenza-like illness (ILI) cases among active duty and retired military personnel and their families using an electronic disease surveillance system. As pandemic influenza A (H1N1) (pH1N1) virus spread globally in 2009, the system detected a steady increase in ILI cases as compared to 2008. We sought to determine and characterize such differences between cases reported during the period from Jan 2008-Dec 2009. Alerta DISAMAR is the Peruvian Navy's electronic disease surveillance system currently implemented in 121 reporting units nationwide. Reporting of ILI cases by age group was carried out weekly via Internet and/or telephone. We retrospectively reviewed ILI cases reported during 2008-2009 and compared cases by year using the Mann- Whitney's U test. P-values <0.05 indicated statistical significance. During the period from 2008-2009 a total of 5469 ILI cases were reported to Alerta. A total of 2282[41.73%] and 3187[58.27%] cases occurred in 2008 and 2009, respectively ($p$=0.02). Analysis by month between 2008 and 2009 showed differences in May and June [pandemic awareness in Peru] ($p$<0.01 and $p$=0.02). The number of cases reported in 2009 (n=1526) in persons 17 years old and under was significantly higher than those reported in 2008[n=856] ($p$<0.01). The analysis also showed significant differences in Lima [1643 vs. 2443, 2008 vs. 2009] and Iquitos [168 vs. 339] ($p$=0.032 and $p$=0.017). Our findings showed a difference between the ILI cases reported in this period, particularly among persons under 17 years of age. We may attribute this difference to pandemic periods distorting both case reporting patterns among stakeholders in surveillance systems, and health care seeking behavior among populations. Public health professionals utilizing surveillance systems should be cognizant that over reporting may occur during similar situations. Therefore, they should aim to confirm "real" cases from those that may be influenced to seek medical care.

## 1139

### DEFINING ELISPOT CUT-OFFS FROM UNREPLICATED TEST AND CONTROL WELLS

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The enzyme-linked immunospot (ELISpot) assay is widely used to detect antigen-specific cytokine-secreting T cells, in particular cell-mediated immune response to vaccines. Various criteria have been used to define positive response, some of them arbitrary. Rigorous methods have been devised for plate layouts with replication, but often this is not present. We present a method for selecting cut-offs which requires negative control wells but not replication. The method uses the proportion of plates in which the number of spot forming units in the test well exceeds that in the control well by a certain amount, rather than the same size difference the other way round. If this proportion exceeds 50% by more than sampling error we can infer that the assay is detecting a signal. Moreover, plotting this proportion, and its confidence interval, against the size of difference suggests the most powerful cutoff to use. We illustrate the method using data from a community-based study of influenza transmission in Vietnam. The complete proteome of H3N2, the haemagglutinin and neuraminidase of H1N1 and the haemagglutinin of H5N1 were represented as peptides of 9-20 amino acids. Preliminary results are available from blood samples of 751 residents aged 5-84

years. Among the peptides with a strong signal was matrix protein: 92 samples had a difference of more than 2 between square-root-test and square-root-control counts, and, of these, 79% had the test well larger than control, rather than the other way round, suggesting a strong signal. By comparison, acidic polymerase, for example, had 47 samples with differences of this magnitude, but about equal proportions of these had test greater than control (47%) and the other way round (53%), suggesting low discriminatory power. The assay has since been refined and we will also present analysis of 1317 paired results based on two different reader settings. This approach should prove useful for those experiments in which testing a greater number of different peptides is preferred to a smaller number of peptides with replication.

## 1140

### BCG STATUS IN NIGERIAN CHILDREN WITH TUBERCULOSIS

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Currently there is only one vaccine against tuberculosis available worldwide: Bacille Calmette-Guérin (BCG). This vaccine, used since 1921, can protect children from severe forms of tuberculosis. There are however varying reports in different countries about the efficacy of BCG in preventing pulmonary TB in children. To evaluate the effect of BCG in preventing pulmonary tuberculosis, 191 HIV negative children aged between 8 months and 14 years with clinical and radiological diagnosis of tuberculosis, receiving antituberculous therapy at a government facility were studied. 95 of them had received BCG and 96 had not. Sixty five percent in BCG group and 76% and in non BCG group were aged over 5 years, while 32% and 20% were 1-5 years old respectively. The others in each group were infants. The commonest presentation was Pulmonary TB in 80% in BCG group and 76% in the non BCG groups, 15% and 20% of children had disseminated TB, while cervical adenopathy was seen in others. Tuberculin skin tests were negative in 34% and 60% in these categories respectively. Only about 50% of children in this series received BCG, but there was not much difference in proportions of children that had pulmonary TB or disseminated TB in both groups. Negative tuberculin test in up to one third of children receiving BCG is noted. Due to the upsurge in incidence of pulmonary tuberculosis locally and globally, there is need to further investigate and review current recommendations for BCG vaccination in this region as well as consider other vaccine candidates that would be effective against most forms of childhood Tuberculosis.

## 1141

### ARE PARAGONIMIASIS AND OTHER PARASITIC INFECTIONS MISDIAGNOSED AS TUBERCULOSIS IN RESOURCE LIMITED AREAS?

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Paragonimiasis (PG) is a food-borne zoonosis caused by trematode worms. This and other parasitic or fungal agents cause infection that predominantly affects the lungs with clinical symptoms similar to tuberculosis (TB) (Vidamalya et al., 2009). Due to the lack of awareness of local health professionals to parasitic agents, they are little evoked in face of chronic cough simulating pulmonary TB. TB offends PG and other parasitic infections by reason of their flagrant clinicoradiologic similarities, thus they are least considered in the face of a chronic cough simulating pulmonary TB, as reported previously. The objective of this study was to estimate the prevalence of PG and other parasitic or fungal causes of respiratory diseases in suspected TB patients. Sputum samples were collected from all patients reporting to the TB clinic of the hospital for the first time. After patients consent, three sputum samples were collected, pooled and processed within 24 hours of collection by: wet preparation with 3% NaOH concentration and microscopy of sediment for parasites and ZN staining of sediment for Acid fast bacilli (AFB). Ethical approval was obtained for this study. 70 patients were recruited within the study period. 44 (62.9%) were males. The mean age was 44.9 years (95% CI: 40.1-49.5). 16 (22.9%) samples were positive for parasitic agents by wet preparation, 2 (2.9%) by ZN staining and 17 (24.3%) were AFB positive. Sputum collected were grouped into four: blood stained, 4(5.7%); muco-purulent, 6(8.6%); muco-salivery, 57 (81.4%) and salivery, 3 (4.3%). 15 (21.4%) of the study patients were HIV/AIDS positive. Parasites identified in wet preparation were Paragonimus *uterobilateralis* (PU) (2/70), Strongyloides *stercoralis* (SS) (1/70) and fungal elements (13/70) of which 8/13 were HIV/AIDS patients. Cryptosporidium parvum (2/70) were found in the ZN staining. Apart from SS, all other parasites were identified in AFB negative patients. Both PU were identified in blood stained samples. The prevalence of PG, fungal infection, Cryptosporidiosis and Strongyloidiasis were 2.9%, 18.6%, 2.9% and 1.4% respectively.These were lower compared to studies carried out in parts of Nigeria and Cameroon, as reported previously. Our sample size might have contributed to this. Our study shows that parasitic infections of the lung are clearly being misdiagnosed as TB; there is the need for awareness to be created among local health care providers.

## 1142

### MODELING CATCH-UP GROWTH FOLLOWING DIFFERENT DRUG TREATMENT REGIMENS FOR ENDEMIC SCHISTOSOMIASIS

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Schistosomiasis is a chronic parasitic infection that results in persistent inflammation that can cause growth stunting and nutritional wasting among affected children. We develop a mathematical model of early childhood development (ages 0-20 years) and the effect of chronic helminth infection on some basic developmental indicators (height, weight). The model was calibrated using the available developmental data in the US (CDC/NCHS), along with infection data and developmental statistics collected in Kenya. We utilized our calibrated model to examine and predict long term outcomes of different age-targeted treatment control strategies. Our results demonstrate the need for early treatment and repeated coverage through the primary school years in order to prevent the under-recognized but disabling sequelae of stunting and undernutrition.

## 1143

### PARASITOLOGICAL AND SEROLOGICAL OF SCHISTOSOMIASIS IN TWO COMMUNITIES ALONG THE VOLTA BASIN

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Schistosomiasis is an important health problem in many developing countries including Ghana. Humans living in schistosomiasis endemic areas develop anti-parasitic antibody responses,which may play distinct roles in immunity. Insight regarding humoral responses in the pattern of *Schistosoma haematobium* and *S. mansoni* infections in any endemic locality is relevant in understanding the pathogenesis of schistosomiasis. This study estimated prevalence of Schistosomiasis in five hundred and eighty three participants aged 5-90 years in Klamadaboe and Torgome. Participants were interviewed to gather Schistosomiasis-related information and screened for *S. haematobium* and *S. mansoni* eggs by microscopy through urine filtration and Kato-Katz technique. ELISA technique was used to confirm infection status of 115 microscopy negative and positive participants to detect anti-IgM and IgG against soluble adult worm and egg antigens.Prevalence of *S. mansoni* and *S. haematobium* infections by microscopy was (17.0%, 10.1%) (N= 159) in

Klamadaboe and (2.6%, 4.7%) in Torgome (N = 424) respectively. Out of 583 participants tested, (10.1%, 39.0%) were positive for haematuria and proteinuria in Klamadaboe and (12.3%, 32.1%) in Torgome. Serum anti-IgM and IgG was detected in (60%, 51%) of people negative for *S. mansoni* eggs (N= 105) and (68%, 54%) respectively for *S. haematobium* (N=112). Fifty nine percent (N =115) had mixed infections with antibodies against both infections. A total of 3.94% (N=76) and 52% (N=27) previously treated participants were positive by microscopy in Torgome and Klamadaboe respectively. Serum antibody detection was useful in confirming infected and uninfected individuals.

## 1144

### SUPPRESSION OF IMMUNOPATHOLOGY IN EXPERIMENTAL MURINE SCHISTOSOMIASIS BY INTERLEUKIN-18-TARGETED FUSION TOXIN, $DAB_{390}$IL-18 I-STUDIES OF *IN VITRO* AND *IN VIVO* EFFICACY

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Schistosomiasis causes pathology in an estimated 200 million individuals. Clinical disease is caused by a complex immunopathologic response to parasite ova, which are deposited in the host tissues. This immunopathologic response is initiated and caused by Antigen Presenting Cells (APC) which express the high affinity IL-18 receptor (IL-18R). DAB390IL-18 is a diphtheria toxin IL-18 fusion toxin protein which functionally inactivates or kills cells which bear the high affinity IL-18R. DAB390IL-18 has been used for the prevention of murine Experimental Auto-immune Encephalitis (EAE). Therefore, we reasoned that DAB390IL-18 might suppress immunopathology in schistosomiasis. In these studies we assessed the *in vitro* and *in vivo* effects of DAB390IL-18 on the development of immunopathology in murine schistosomiasis. DAB390IL-18 suppressed IL-18, lectin mitogen (Con A), and soluble *Schistosoma mansoni* egg antigen -induced lymphocyte proliferation and *in vitro* granuloma formation. In addition, DAB390IL-18 suppressed *in vitro* IL-18R expression. DAB390IL-18 also suppressed the development of granulomas and collagen deposition *in vivo* in the livers of infected animals. Therefore, DAB390IL-18 may have potential for the targeted reduction of immunopathology due to schistosomiasis in man

## 1145

### THE ASSOCIATION BETWEEN SCHISTOSOME INFECTION, ATOPY AND AUTOREACTIVITY IN A ZIMBABWEAN POPULATION

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In animal experimental models, parasitic helminth infections can protect the host from atopy and autoimmune diseases. We have conducted the first population-scale human study investigating the relationship between helminth parasitism and autoimmune-reactivity. In addition we have conducted a study of the relationship between atopy and infection intensity in 2 villages of differing schistosome infection intensity. In people naturally exposed to the blood fluke helminth parasite causing schistosomiasis, we found that autoimmune-reactivity and atopy is inversely associated with current infection intensity but is independent of host age, sex and HIV status. Autoimmune-reactivity increases 6 months after anti-helminthic treatment. The implications of these findings are relevant in understanding both the aetiology of autoimmune diseases and in predicting the long-term consequences of large-scale schistosomiasis control programs.

## 1146

### THE APPLICATION OF INTEGRATED MODEL OF S LANDSCAPE PATTERN ANALYSIS AND BAYESIAN MODELS ON SCHISTOSOMIAIS CONTROL

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With the ecological environment changing, including climate warming and human activities, the prevalence of schistosomiasis japonica will also change. The aim of this study is to establish an integrated model to evaluate and predict the change of the endemic status of schistosomiasis before and after the project of land conversion for restoration in the Dongting Lake region. The data collected was the sampling survey on schistosomiasis from 1995 to 2006 in Hanshou County in Hunan province, P.R China. Normalized difference vegetation index (NDVI) and Wetness, land surface temperature (LST) and landscape factors (land-use/type) were extracted from remote sensing images. Landscape metrics were calculated from land-use/type images. Spatio-temporal Bayesian models based on landscape analysis and the sensitivity/specificity of diagnostic test(s) were established to understand and predict the spatio-temporal pattern of schistosomiasis. The results showed that the change of spatial structure each year during 1996 and 2005 were significant. The negative correlation of the prevalence of *S.japonicum* infection and NDVI were significant as well. The prediction map of 2002 showed the whole prevalence of *S.japonicum* infection was low, and the areas whose prevalence was more than 1% were mostly along bodies of water such as the Muping Lake and Yuanshui River. The average prediction prevalence was about 2.22% in 2005, and these areas were also along bodies of water. The changing map of *S.japonicum* infection prevalence in the southern areas between 1996 and 2005 were not found to be significant. However, the prevalence increased significantly in the northern areas. The impact of the project, land conversion for restoration on the prevalence of *S.japonicum* was significant, with the impact of partial abandonment for restoration being stronger than the complete abandonment part of the project.

## 1147

### CURRENT EFFICACY OF PRAZIQUANTEL AGAINST *SCHISTOSOMA JAPONICUM* INFECTION: A FIELD EVALUATION IN MAIN ENDEMIC FOCI OF CHINA

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Praziquantel is widely used for the treatment of human schistosomiasis. However, in recent years, there is a increasing concern about the resistance of *Schistosoma* species to praziquantel. Under laboratorial conditions it is possible to induce resistance of *S. mansoni* to praziquantel with multiple sub-curative doses, and a decreased sensitivity of *S. mansoni* to praziquantel has been found in many endemic areas. There are also several schistosomiasis cases caused by *S. hematobium* in whom repeated standard failed to clear the infection reported. Since 1992, the World Bank Loan Project for Schistosomiasis Control initiated in China, praziquantel-based chemotherapy has been conducted to control the morbidity and reduce the prevalence and intensity of *S. japonicum* infection. After extensive, long-term repeated praziquantel chemotherapy, whether there is decreased efficacy of praziquantel against *S. japonicum* is paid many attentions. The study we described here was designed to evaluate the efficacy of praziquantel against *S. japonicum* in main endemic foci of China. During the non-transmission period of schistosomiasis, a random sample of 4760 subjects from 11 villages of 5 provinces in China that are endemic for *S. japonicum* were examined using the miracidium-hatching method for detection of the stool samples, and a total of 584 subjects were identified as being infected with *S. japonicum*, with a prevalence rate of 12.27%. Among them, 565 stool-egg-positive subjects were treated with praziquantel in a single oral dose of 40 mg/kg. Six weeks post-treatment, among the 505 villagers re-examined, 480 (95.05%)

had no detectable *S. japonicum* eggs. Twenty-one subjects still excreting eggs after the first treatment were treated with praziquantel for the second time. All stool samples, including those from those participants with second treatment were re-examined six weeks after the second treatment, and no stool-egg-positives were found. The results indicate that the efficacy of praziquantel against *S. japonicum* is still high, which has not changed after more than two decades of repeated, expanded chemotherapy in main endemic areas of China. It is suggested that no evidence of tolerance or resistance of *S. japonicum* to praziquantel is detected in China.

## 1148

### CD23B MRNA EXPRESSION DOMINATES OVER CD23A MRNA EXPRESSION IN B CELLS FROM *SCHISTOSOMA MANSONI*-INFECTED OR UNINFECTED ADULTS IN WESTERN KENYA

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IgE is associated with resistance to schistosomiasis and exerts its functions through its receptors, the high affinity receptor FcεRI and the low affinity receptor FcεRII (CD23). We previously demonstrated that expression of CD23 by circulating B cells correlates with the development of resistance to schistosome infection in a multiply treated cohort in an endemic area in western Kenya. There are two isoforms of the 45-kD CD23; CD23a and CD23b, which differ only in their cytosolic domains. CD23a is constitutively expressed by B cells and functions in the endocytosis of CD23-IgE antigen complexes, but the role of CD23b, which is IL-4-inducible, in B cells is not known. The objective of this study was to define the role of CD23 in generating immunity to *S. mansoni* re-infection. B cells were isolated from the peripheral blood of occupationally-exposed adult males assessed for expression of CD23a and CD23b and compared to uninfected cohorts. CD23a is the dominate isoform in peripheral blood B cells from uninfected/unexposed subjects. In contrast, CD23b was found to be elevated relative to CD23a in B cells from both *S. mansoni* infected and non-infected endemic controls. Functionally, IL-4-generated CD23b+ B cells appear to display altered B cell differentiation pathways. These results suggest that CD23b has functional significance for B cells and studies are underway to define the role of CD23b+ B cells in schistosomiasis.

## 1149

### HUMAN MONOCYTE EXPRESSION AND ACTIVITY IN *SCHISTOSOMA HAEMATOBIUM* IMMUNE RESPONSES

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Monocytes derived from myeloid bone marrow precursors circulate in the blood before entering tissues and maturing into macrophages with a distinct phenotype dependent on the cytokine signals received. Macrophages and monocytes that are stimulated with Th1 cytokines, IFNg and TNFa, develop a classically activated phenotype, catalysing L-arginine to nitric oxide in response to microorganisms. In contrast, Th2 stimulated monocytes develop into alternatively activated monocytes and macrophages (AAM), characterised by a metabolic pathway in which L-arginine is converted into L-ornithine and urea leading to the suppression of T cell proliferation. AAM are associated with wound healing, controlling inflammatory immune responses and parasitic infections. While classically activated macrophages and monocytes (CAM) behave similarly in murine and human systems, AAMs do not seem to have such an overlap, and indeed the characteristic murine marker genes for AAM are notably absent in the human genome. However, an alternatively activated genotype in human filarial infections, with an upregulation of the arginase encoding gene ARG-1 has been reported previously. Using PBMCs isolated from a cohort of 200 participants in a *Schistosoma haematobium* endemic area in rural Zimbabwe we investigated the influence of infection on the phenotypic and genetic expression patterns in human monocytes, identifying markers for alternative activation. Furthermore the arginase expression patterns were elucidated and associated with liver pathology and infection intensity.

## 1150

### COMPATIBILITY PATTERN OF *BIOMPHALARIA ALEXANDRINA* SNAILS IN WATER COURSES OF ALEXANDRIA GOVERNORATE, EGYPT

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Schistosomiasis is one of the ten tropical diseases specially targeted for control by the World Health Organization. Despite the major advances in the control of the disease, yet the transmission of the disease shows little evidence of slowing down globally and continues to spread to new geographic areas.

Our aim was to determine the compatibility pattern of Biomphalaria alexandrina snails collected from two districts representing eastern (Abees) and western (Alamreya) areas of Alexandria governorate. This will pave the way for further epidemiological and biological control studies of the intermediate host of Schistosoma mansoni. The results denoted that the natural infection rate of the snails from the field of the two areas was 2.3 and 3.3% respectively. At the age of 4-6 weeks, three hundred snails of the first generation of the field snails (laboratory adapted snails) from each group, were exposed to eight miracidia of the local strain of S. mansoni. Infection rate of the laboratory adapted snails of both groups was 15.67 and 18.33% respectively. Total cercarial production/100 exposed snails were 27484.56±8828.47 and 32937.33±10315.83 while the prepatent period was 31.96 and 30.58 days. No statistical significant differences in the results were present between both groups. By using Random Amplified Polymorphic DNA (RAPD-PCR) technique, differentiation between susceptible and resistant snails was achieved. Bands of resistance as well as bands of susceptibility could be detected by five out of eight primers used. This technique was proved to be easy, applicable and does not need previous information of nucleotide sequences.

## 1151

### NATIONAL SEROLOGICAL SURVEY OF *HAEMATOBIUM* SCHISTOSOMIASIS IN MOROCCO: EVIDENCE FOR ELIMINATION

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The Moroccan Public Health Ministry launched a process of eliminating schistosomiasis in 1994. From 2005 to 2009 the epidemiological situation was marked by interruption of transmission at the national level, with only a few residual cases recorded. Our present study is the first systematic serologic survey to evaluate the transmission status in remaining endemic foci. Two thousand three hundred and eighty two children, born after the date of the last autochthonous cases, were selected from provinces with histories of high schistosomiasis transmission: Errachidia, Tata, El Kelaa Des Sraghna, Chtouka Ait Baha, and Beni Mellal. Specific antibodies directed to *Schistosoma haematobium* adult worm microsomal antigens (HAMA) were targeted using an enzyme-linked immunoelectro-transfer blot (EITB) assay. The results showed an absence of antibodies in all the sera. Consequently, our findings confirm a very low transmission status or a likely interruption of haematobium schistosomiasis transmission within these last endemic hot-spots.

## 1152

### PROFILE OF SPECIFIC HUMORAL RESPONSES AGAINST SCHISTOSOMIASIS IN AN ENDEMIC RURAL AREA OF MINAS GERAIS STATE, BRAZIL: A LONGITUDINAL STUDY

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We examined the pattern of specific IgE and IgG4 against *Schistosoma mansoni* antigens (SEA or SWAP) of residents of an endemic area in Brazil. Parasitological and serological analyses were performed in 152 individuals who participated in the entire period of evaluation. The follow up consisted of collection of three serum and fecal samples (time zero, one and three years after treatment), in the period between 2001 and 2006. Before treatment, the prevalence of *S. mansoni* was 57.9% (CI95% 50.06-65.74) and the geometric mean egg counts 64.1 (CI95% 52.67-75.51). After treatment, the prevalence decreased significantly to 15.1% (CI95% 9.41-20.79) and 27.6% (CI95% 20.50-34.70) in 2002 and 2006, respectively. The intensity of infection was significantly reduced to 37.8 (CI95% 35.07-40.55) and 36.4 (CI95% 33.93-38.89) eggs/g after treatment. Analysis of IgE-SEA demonstrated that significant differences in antibody production was only detected after three years of treatment, with infected individuals presenting higher anti-SEA IgE levels when compared to those egg-negative. Although differences in anti-SWAP IgE production were observed among infected and egg-negative individuals, they were limited to the initial evaluation before treatment. Anti-SEA and SWAP IgG4 levels were significantly higher in infected individuals before and after treatment, when compared to egg-negative individuals. Significant association was observed only to SEA-specific antibodies at time zero, showing a correlation between intensity of infection and IgE ($r = -0.266$, $P = 0.012$), and also between geometric mean egg counts and IgG4 ($r = 0.239$, $P = 0.025$). Similar association was observed between parasite burden, IgG4 to SWAP ($r = 0.502$, $P = 0.015$) and parasite burden and IgE to SEA ($r = 0.470$, $P = 0.002$), one and three years after treatment, respectively. Our results may contribute to the evaluation of the use of specific IgE and IgG4 ELISA to predict susceptibility/resistance to human schistosomiasis. Analysis to evaluate the association between antibody production and infection is under investigation.

## 1153

### LONG TERM INFECTIONS; GENE EXPRESSION PROFILES OF SCHISTOSOME/SNAIL COMBINATIONS

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Oligo-based microarrays bearing features derived from either *Biomphalaria glabrata* or *Schistosoma mansoni* are probed with transcribed sequences derived from whole bodies of snails with *S. mansoni* infections of varying age. This will enable measurement of response profiles from both host and parasite a) at 3 weeks post-infection (wpi), before cercariae production and release (referred to as "shedding") commences; b) early in the interval of cercarial shedding (about 5 wpi); and c) at a time following more prolonged periods of cercariae shedding (8 wpi). It will be determined if immune or other genes in snails undergo pronounced up- or down-regulation before or after cercariae production, and if parasite components, potentially including factors like innate immune pathway or stress-related proteins that could contribute directly to protection of the infected snail, or that could modulate snail defense responses, are significantly altered in their expression during the same time points. An understanding of which transcripts from snail host or schistosome parasite, separately or combined, prolong the life of cercariae-shedding snails may provide inroads for control aimed at reduction of release of cercariae (infective for humans) by infected Biomphalaria snails.

## 1154

### DIVERSIFICATION OF SNAIL RESPONSE FACTORS TO PARASITE INFECTION, EXCEPTION OR TREND?

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Microarrays incorporating sequences expressed by *Biomphalaria glabrata* exposed to various immune challenges (wounding, bacteria, exposure to schistosomes) were used to identify candidate immune response factors from both BB02 (susceptible to S. mansoni) and BS-90 (resistant) *B. glabrata* laboratory strains at 12h, 24h and 120h post exposure to bacteria or schistosome parasites. Known immune factors as well as putative immune factors among novel (unknown) sequences were selected for analysis to expand insights into immunity of B. glabrata, especially transcripts that are held in common among all responses, as well as those that are unique to schistosome challenge. Five groups of candidate defense genes will be characterized by full-length sequencing. In light of indicated antigenic variation by schistosomes of mucins and of somatic diversification of fibrinogen-related proteins (FREPs), a category of snail immune lectins, it becomes highly relevant to investigate whether the occurrence of diverse or diversified immune factors in an invertebrate host that contributes to parasite transmission like the snail *B. glabrata* is more common than previously assumed. Accordingly, results from application of SSCP and from sequence comparsions of multiple cloned cDNA amplicons of putative non-self recognition factors (including FREPs and other lectins) will be presented.

## 1155

### CROSS-SECTIONAL AND LONGITUDINAL STUDY ON SCHISTOSOMIASIS AND ITS RELATIONSHIP WITH SOCIOECONOMIC VARIABLES IN A RURAL AREA IN MINAS GERAIS STATE, BRAZIL

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The objective of this study was to identify the socioeonomic determinants of schistosomiasis in a hyperendemic rural area in Minas Gerais State, Brazil, during the period 2001-2009. The study population comprised 528 persons in 2001 and 533 in 2009, including 389 individuals who lived in Virgem das Graças at these two points in time. Socioeconomic and water contact data were collected in household surveys. All participants were examined parasitologically in 2001, 2005 and 2009 and treated with praziquantel. The results showed a reduction in prevalence from 57.7% to 26.5% and in geometric mean egg counts from 57.98 (CI95% 55.84-60.11) in 2001 to 13.45 (CI95% 11.29-15.60) in 2009. *S. mansoni* infection was significantly correlated with ownership of motorized vehicles, electricity, latrines and water storage tanks ($p<0.001$). The number of houses using the streams decreased during those 8 years, largely as a result of increased use of piped water from shallow wells and springs ($p<0.001$). In the multivariate logistic regression model *S. mansoni* infection in 2009 was significantly correlated with age group 10-19 years (OR=1.18 CI95% 1.04-1.34) and well ownership (OR=1.09 CI95% 1.01-1.18) and faucet (OR=1.25 CI95% 1.06-1.47). Self evaluation of family health conditions showed that 30.0% of all households attributed health improvements during the study period to increased income from the government's "Bolsa Familia" social program, which benefits poor families and was implemented in 2004. The finding that improved socioeconomic conditions were associated with increased use of a safer water supply and declines in *S. mansoni* infection warrants further studies in other communities.

## 1156

### INVESTIGATION OF SCHISTOSOMIASIS TREATMENT ROTATION STRATEGIES TO MAKE THE MOST OF EXPENSIVE PRAZIQUANTEL

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Schistosomiasis is a parasitic disease affecting 200 million people worldwide, and about 35 million in Nigeria alone. The drug praziquantel (PZQ) effectively combats the disease, and is used in annual mass treatment programs targeting the urinary and intestinal forms of the disease, *S. haematobium* [SH] and *S. mansoni*. When it is not donated, the cost of PZQ (about $0.20 per treatment) is a major constraint to scaling up treatment programs in countries that need it most. To maximize impact, the State Ministries of Health Delta, Nasarawa and Plateau States, assisted by The Carter Center, have assessed the feasibility of PZQ treatment rotations (drug holidays). Mass drug administration (MDA) with PZQ was provided in villages with >20% prevalence of SH (determined through rapid reagent strip test for hematuria in school children). A strategy has been adopted wherein targeted communities in endemic local government areas (LGAs) receive annual PZQ treatment for three to five years followed by a "drug holiday." MDA was rotated to other LGAs while health education and monitoring for recrudescence among school children continued in sentinel villages in LGAs on "holiday." From 2004 - 2007, prevalence data has been collected in communities after three to five years of annual treatment, and during treatment holiday. Communities treated for three years recrudesced in two years to an average of 24.5%, exceeding the 20% threshold for MDA, suggesting that retreatment was necessary. In contrast, those receiving four years of treatment experienced average recrudescence ranging from 3.6% (Delta state) to 13.2% prevalence (Plateau and Nasarawa States) after 2 years. Those receiving five years of treatment recrudesced to 11.6% prevalence after 2 years. We concluded that four to five years of annual treatment prior to holiday is the best rotation strategy in terms of the speed of disease recrudescence and the need for retreatment.

## 1157

### SCHISTOSOMIASIS IN MINIGO, TANZANIA: A STUDY ON PREVENTIVE BEHAVIOR AND ITS CORRELATION TO DISEASE PREVALENCE

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Schistosomiasis, the second-most prevalent parasitic disease in humans, manifests as a granulomatous inflammatory response to *Schistosoma* eggs, primarily within hepatic and intestinal systems. We evaluated the efficacy of preventive behavior for schistosomiasis within the village of Minigo, Tanzania (pop. 3635). Daily activities with infested waters in this endemic Lake Victoria Region put villagers at risk for contracting the trematodes (*S. mansoni* and *S. haematobium*). A comprehensive on-site screening and preventive education program produced stool and urine samples. Light microscopy was used to verify schistosomes. Test positive subjects were given a physical exam and treated with Praziquantel. 13.1% of participants (n=229, m=136, f=93) were test positive, fishermen composing the largest proportion (5.7%). Approximately half (n=110) of the subjects participated in an oral questionnaire and reported preventative (45.5%) and non-preventative (54.5%) behavior. Of the test positive cases in this subgroup, 33.3% reported preventative behavior and 66.7% reported no preventative behavior, a difference of two fold. Individual prevention was assessed with three criteria: previous testing, previous schisto study participation, preventive behavior. Although the three largest subgroups were students, farmers, then fishermen, results displayed an inverse relationship with prevention. Finally, ROC analysis, used in the cost/benefit analysis of diagnostic decision-making, was applied to subjects with respect to an understanding of schistosomiasis. Subgroups displayed a high sensitivity (0.78-1) for risks and awareness of infection, but overall low specificity (0.04-0.32), which is interpreted as poor understanding of the disease factors and risks. Data show preventative behavior reduces schistosomiasis prevalence. Regular extension of services from point-of-care facilities to communities is a preventive approach, which would encourage compliance to preventive intervention and treatment and may facilitate an increase in ROC specificity and an overall reduction of prevalence.

## 1158

### THE ROLE OF CD11B+ MONONUCLEAR PHAGOCYTES IN SCHISTOSOME DEVELOPMENT

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Worldwide, over 200 millon people are infected with schistosomiasis. Importantly, transmission and pathology of the disease is dependent on worm maturation. Earlier, we demonstrated that naïve CD4+T cells facilitate worm development in the absence of TCR signaling. We further correlated the presence of naïve CD4+T cells with changes in steady-state expression of genes required for monocyte/macrophage maturation. Interestingly, direct stimulation of mononuclear phagocytes in the absence of CD4+T cells restores worm development. Hence, we hypothesize that schistosome development is dependent on innate immune signals, which are facilitated by the presence of naïve CD4+T cells. Recent studies have implicated a role for naïve T cells in priming dendritic cell maturation through the interaction of B7-H1 on resting T cells. Here, we further hypothesize that resting naïve CD4+T cells prime monocyte/macrophage maturation through B7-H1-PD1 signaling, and that this interaction is required for the appropriate innate immune signals that direct worm development. To support this hypothesis, we show that inert OVA-specific CD4+T cells, expressing B7-H1, directly interact with CD11b+ mononuclear cells in OTII-RAG-/- transgenic mice. Moreover, during the course of a schistosome infection, CD11b+ cells segregate into two distinct populations (CD11b+Hi and CD11b+Lo) that differ in their scatter properties and relative expression of Ly6C, CD115, and PD1. Remarkably, the relative ratios of these populations significantly differ between OTII-RAG-/- mice and RAG-/- mice, which lack T cells. Specifically, RAG -/- mice accumulate a larger proportion of CD11b+Lo cells that express less CD115, which is required for monocyte/macrophage maturation, and less PD1, which is a receptor for B7-H1. This evidence suggest that there is inadequate maturation of macrophages in the absence of naïve CD4+T cells, which correlates with the lack of schistosome development, and thus indicates that naïve CD4+T cells indirectly facilitate worm development by modulating monocyte/macrophage function.

## 1159

### OROPOUCHE FEVER VIRUS: MOLECULAR EPIDEMIOLOGY AND EVOLUTION

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Oropouche virus (VORO; *Bunyaviridae, Orthobunyavirus*) is one of most important arbovirus which infects humans in the Brazilian Amazon, and is also the causal agent of Oropouche fever. Between 1961 and 2009, dozens of epidemics were registered in several urban centers of the Brazilian states of Acre, Amapá, Amazonas, Maranhão, Pará, Rondônia and Tocantins, and also in Panama, Peru and Trinidad & Tobago. This work aimed to develop a retrospective epidemiologic and molecular study of

VORO emphasizing its distribution, epidemic dynamics in the period, as well as the dispersion of the VORO genotypes in Brazil and other Latin American countries as a contribution to understanding of the molecular epidemiology of it. A total of 66 VORO isolates of the Instituto Evandro Chagas collection were growth into VERO cells and suckling mice; then, RNA was extracted and cDNA prepared by RT-PCR; the amplicons were purified and submitted to nucleotide sequencing to further molecular and evolution analyzes including genetic reassortment, molecular clock and viral dispersion. It was demonstrated the circulation of four different genetic lineages of VORO in the Brazilian Amazon (genotypes I, II, III, and IV); the genotypes I and II were respectively the most distributed VORO genotypes in Occidental and Oriental Amazon areas. These and the genotype III have been continuously under evolution pressure and changing by the mechanism "boom and boost" which result in an emergence of new VORO sub-genotypes that replace the older circulating sub-lineages in an area. The genotype III which was previously recognized in Panama was identified in the Amazon and Southeast regions. The results obtained by the comparative phylogenetic analyses of the SRNA and MRNA topologies suggest that VORO uses the genetic reassortment as mechanism to further generate its viral biodiversity, and the genotype I is the most stable, while the genotype II is the most unstable, and therefore under higher evolutionary pressure; it was recognized a new VORO genotype in this study, the genotype IV. The molecular clock analysis showed that VORO emerged in Pará state approximately 223 years ago, and along of the years did its dispersal and evolution through the Pan-Amazon as well as to the Caribbean and Central America region, and the genotype I was responsible by the emergence of all other VORO genotypes.

## 1160

### ISOLATION OF H5N1 INFLUENZA VIRUSES FROM WILD TERRESTRIAL BIRDS IN KAZAKHSTAN

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Highly pathogenic avian influenza viruses of subtype H5N1 were identified in Southeast Asia in 1996 and have spread in recent years across broad regions of Eurasia and Africa. These viruses have been shown to be highly lethal in chickens and other poultry species as well as several types of aquatic birds, however, there are only limited reports of H5N1 surveillance in terrestrial birds. In this study, we examined the presence of H5N1 in wild terrestrial birds in Kazakhstan. Wild birds were caught at the ornithological station "Chockpack" in the Zhambylskaya oblast. Cloacal swabs were collected according to accepted FAO standards. Field samples were tested by several methods including RRT-PCR, for H-, and N- genes. During the period 2007-08, cloacal swabs from 993 representatives of wild terrestrial birds in the family's Meropidae, Corvidae, Muscicapidae, Sylvidae, Falconidae, Hirundinidae, Motacillidae, Turdidae, Paridae, Laniidae, Accipitridae, Emberizidae, Upupidae, Strigidae, Sturnidae, Ploceidae, Columbidae, Fringillidae, Accipitridae, Motacillidae, Prunellidae, Caprimulgidae, Cuculidae, Tytonidae, and Fringillidae were collected. Of the 993 samples, 44 (4.43%) positive samples for type A influenza viruses including 28 (2.8%) positive samples from the birds of the Columbidae family (pigeon), 5 (0.5%) positive samples from birds of Corvidae family (jackdaw, rook), 4 (0.4%) positive samples from birds of the Ploceidae family (sparrow), 3 samples (0.3%) from birds of Meropidae family (golden bee-eater), 2 samples (0.2%) from birds of Hirundinidae family (swallow), 1 sample (0,1%) from birds of Sturnidae family(starling) and 1 sample (0.1%) from birds of Turdidae family (blue throated robin). Seven strains of subtype H5N1, including 3 from pigeons (Columbidae family), 2 from golden bee-eater (Meropidae family), 2 from starlings (Sturnidae family) and 1 from a rook (Corvidae family) were detected. The data support that AI viruses of H5N1 subtype circulate among wild terrestrial birds which live close to humans and domestic birds in Kazakhstan.

## 1161

### ACUTE HEPATITIS E IN IMMIGRANTS AND NATIVE PATIENTS IN VICENZA, ITALY

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Hepatitis E (HEV) virus is the most common cause of acute viral hepatitis in developing countries, but is gaining increasing global attention. Hepatitis E is generally self-limiting but in pregnant women and patients with chronic liver disease death can occur. In non-endemic countries, the infection occurs in sporadic cases: a zoonotic transmission involving pigs and other mammalians has been demonstrated.

We report our experience on HEV acute hepatitis in the Infectious and Tropical Diseases Department of the S.Bortolo Hospital in Vicenza, Italy. From 1995-2010, 20 cases of acute hepatitis E (17 male and 3 female, none pregnant, mean age 30 + 4.9 y) were admitted to our Department. The diagnosis was made by the detection of anti-HEV IgM together with HEV-RNA in blood and in stool. Four patients had recently immigrated to Italy (mean stay 19 days), 14 patients acquired the infection after travelling to their country of origin (15 Bangladesh, 4 India and 1 Pakistan) after several years of residence in Italy (mean 9.13 ± 2.3 years). Only one patient, an immigrant from Bangladesh who had been living in Italy for 9 years, had no history of recent travels. However, he was a household contact of another patient with HEV infection (secondary case). Only in one Italian male no travels and contacts with hyperendemic areas could be found. In this case, genotype 3 of HEV can be assumed, while all the other cases had genotype 1 (Burmese). All patients had a self-limited icteric illness (mean bilirubin level 8,6 ± 6.44 mg/dl, mean ALT level 2837,6 ± 1559 UI/l). None of the patients had pre-existing chronic liver disease. In conclusion, HEV infection is generally an imported disease in our town, but secondary and the odd autochthonous case can occur. The number of cases reported slightly increased since 2004. (1,1 cases/y in 1995-2003 vs 1,8 in 2004-2009). In particular, the infection is more common in immigrants travelling to the country of origin after staying in Italy for several years: a loss of previously acquired immunity can be hypothesized in these cases.

## 1162

### MONOCLONAL AND POLYCLONAL ANTIBODIES FOR DEVELOPMENT OF RAPID IMMUNOASSAYS FOR DETECTION OF SAND FLY FEVER VIRUSES

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Various viruses and Leishmania parasites are carried by the sand fly *Phlebotomus papatasi* and cause sand fly fever, or cutaneous and visceral Leishmaniasis. These diseases are designated high on the list of infectious threats to deployed troops. The development of simple field tests to detect and differentiate the causative agents of these diseases directly in sand flies or in humans would be of great importance. Sand Fly Fever is caused by a group of viruses often termed Sand Fly Fever Viruses (SFFV), or Papatasi viruses, or Phleboviruses and is typically caused by e.g. Toscana, Naples and Sicilian viruses which are closely related to Rift Valley Fever Virus (RVFV). The Toscana virus is confined to Italy and the Mediterranean basin, whereas the Naples and Sicilian viruses are more prevalent and often found together in the Middle East through Pakistan and Afghanistan, and recently in Algeria. The Toscana virus can lead to severe meningitis, whereas the Naples and Sicilian virus lead to short term febrile illness. Recently, other viruses related to the Naples virus (Massilia virus, Southern France and Punique, Tunisia) and Sicilian virus (Cyprus virus, Algeria and Utique, Tunisia) have been identified. Serological responses

to the viral Nucleocapsid (N) protein, in particular, specific IgM and IgG responses as detected in ELISA or western blot, are frequently used in SFFV diagnosis. However, high degree of cross reactivity between different SFFV and low antibody titers can complicate differential diagnosis. We have developed polyclonal and monoclonal antibodies to the different SFFV N proteins and have designed several rapid immunoassays for antigen detection either for use in sand flies or in human sera. In this study we describe the use of these antibodies in development of two rapid antigen tests 1) to differentiate SFFVs from Leishmania parasites; and 2) to differentiate Sicilian virus from Naples and Toscana virus. The potential field use of these tests will be discussed.

## 1163

### DIAGNOSTIC PERFORMANCE OF ROTAVIRUS AND NOROVIRUS TESTING ON RECTAL SWAB SPECIMENS: IMPLICATIONS FOR OUTBREAK INVESTIGATIONS

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Diarrhea outbreaks can result in high morbidity and mortality, particularly in children in developing countries. Identifying etiological agents is critical in controlling outbreaks. We evaluated the diagnostic performance of specimens collected using rectal swabs for the detection of rotavirus and norovirus. Patients meeting a diarrhea case definition (≥3 loose stools in 24 hours during the last seven days) were enrolled through an ongoing population-based surveillance system based at a hospital and an ambulatory clinic in the Department of Santa Rosa, Guatemala. From January through March 2009, we attempted to collect a rectal swab and whole stool sample from patients <5 years old with diarrhea. Rectal swabs were placed in phosphate-buffered saline media. Both specimens were kept at 4°C, and tested for norovirus using real time-reverse transcription-polymerase chain reaction and for rotavirus via enzyme linked immunosorbant assay using monoclonal antibodies to detect group specific antigen present in Group A rotavirus. We calculated sensitivity and specificity assuming testing from whole stool samples as the gold standard. We enrolled 102 cases with paired whole stool and rectal swab samples. The median age of patients was one year (range: 0-4 years); 38 (37%) were ambulatory patients and 64 (63%) were hospitalized. Twenty (22%) were positive for norovirus and 56 (55%) were positive for rotavirus. The overall sensitivity for rotavirus testing on rectal swabs was 90% [95% Confidence Interval (95% CI): 79% - 96%] with a specificity of 92% (95% CI: 81% - 98%). For norovirus overall sensitivity was 57% (95% CI: 33% - 79%) and specificity 91% (95% CI: 83% - 95%). Performance for norovirus was highest among children <1 year old with vomiting, with a sensitivity of 71% (95% CI: 36% - 92%) and specificity 92% (95% CI: 74% - 98%). Sensitivity and specificity did not vary significantly among hospitalized or ambulatory patients. In conclusion, sensitivity and specificity for rotavirus testing from rectal swabs were high, but sensitivity for norovirus was lower. Testing of specimens from rectal swabs is a viable alternative to whole stool for detection of rotavirus, particularly during outbreaks where collection of whole stool may be difficult. Norovirus testing from rectal swabs could also be used to confirm the etiology of the outbreak.

## 1164

### ESTABLISHING THE CAPACITY FOR INFLUENZA SENTINEL SURVEILLANCE IN LIMITED-RESOURCE SETTINGS: A PROGRESS REPORT FROM WEST AFRICA

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The detection of highly-pathogenic avian influenza in poultry in West Africa in 2006 led to recognition of the urgent need for widespread sentinel surveillance for influenza. Only Senegal and Côte d'Ivoire were conducting influenza surveillance at this time. Since then, training and laboratory equipment have been provided by Centers for Disease Control and Prevention, NAMRU-3, the US Navy Global Emerging Infections System, the US Department of State, and the Institut Pasteur with the goal of building influenza surveillance capacity based on real-time PCR testing in several other West African countries, both among outpatients with influenza-like-illness (ILI) and among inpatients with severe acute respiratory infection (SARI). Initial assessment visits were conducted in several countries in 2008 and 2009. To summarize current surveillance capability, these assessments and subsequent trip reports were reviewed, and ministries of health were contacted. Influenza surveillance has been conducted in Senegal since 1996, in Côte d'Ivoire since 2003 and in Ghana and Nigeria since 2007. In 2010, the numbers of ILI and SARI sites in these countries were 14/4, 9/8, 15/3, and 4/4, respectively, and they tested a total of 1550, 1382, 3848 and 1408 specimens, respectively, from April 1, 2009 to March 31, 2010. The initial assessment visits were conducted in Benin, Burkina Faso, Liberia, Mali, Mauritania, Niger, Sierra Leone and Togo. Five of these countries were judged to be ready to develop influenza surveillance capacity. They have chosen to begin surveillance with the following number of ILI and SARI sites: Niger 12/12; Mali 5/1; Mauritania 1/1; Burkina Faso 1/1; Togo 1/1. Niger and Mali have recently begun influenza surveillance. Mauritania, Burkina Faso and Togo are expected to begin in the next 2-3 months. In conclusion, significant progress has been made recently in influenza surveillance in West Africa. The capacity of countries has improved and the number of countries with functional surveillance systems is increasing.

## 1165

### PLANNING FOR RIFT VALLEY FEVER VIRUS: USE OF GIS TO ESTIMATE THE HUMAN HEALTH THREAT OF WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*)-RELATED TRANSMISSION

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Rift Valley fever virus (RVFV) is a mosquito-borne phlebovirus of the *Bunyaviridae* family that causes frequent outbreaks of severe animal and human disease in sub-Saharan Africa, Egypt, and the Arabian Peninsula. Based on its many known competent vectors, its potential for transmission via aerosolization, and its progressive spread from East Africa to neighboring regions, RVFV is considered a high-priority, emerging

health threat for humans, livestock, and wildlife in all parts of the world. Introduction of West Nile virus to North America has shown the potential for 'exotic' viral pathogens to become embedded in local ecosystems. While RVFV is known to infect and amplify within domestic livestock such as taurine cattle, sheep, and goats, if RVFV is accidentally or intentionally introduced into North America, an important unknown factor will be the role of local wildlife in the maintenance or propagation of virus transmission. We examined the potential impact of RVFV transmission via white-tailed deer (*Odocoileus virginianus*) in a typical Ohio (northeastern United States) urban-suburban landscape, where livestock are rare, but these potentially susceptible ungulate wildlife are highly abundant. GIS modeling results, based on overlap of mosquito, human, and projected deer densities, indicate that a significant proportion (192 / 458 mi2, or 42 %) of the Cuyahoga County urban and peri-urban landscape could be affected by RVFV transmission during the late summer months. Deer population losses, either by intervention for herd reduction or by RVFV-related mortality, would substantially reduce these likely transmission zones to 20.5 mi2, or by 89%. Integrated strategies for vector and animal control, combined with human population preventive measures, will be essential to prevent establishment of novel arboviral pathogens in this typical North American landscape.

## 1166

### INVESTIGATION OF ARBOVIRAL INFECTIONS OF BIRDS IN SÃO PAULO STATE, BRAZIL

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Arthropod-borne viruses cause diseases of great importance to public health and, for this reason, it is necessary a constant surveillance to detect circulating viruses in a specific area. Among them, West Nile virus (WNV) is an arthropod-borne virus member of the genus *Flavivirus*, family Flaviviridae, which is endemic in Africa and Middle East, but has spread rapidly across Europe and the Americas, causing severe neuroinvasive disease outbreaks in humans and animals. To date, there are no reported cases of WNV isolation in Brazil but a surveillance system has been established by the Ministry of Health, searching for evidence of WNV infections in wild birds captured in different landing sites along the Brazilian territory. Thus, due to the importance of this emerging disease and the lack of a recent epidemiological study of arbovirus circulation, other than dengue and yellow fever, in the state of São Paulo, this study investigated the evidence for West Nile and other arbovirus infections in wild birds captured in the state. Birds were captured in three well established landing sites throughout the state of São Paulo: Central region (21°41'06"S/48°05'03"W), Northwest (20°52'20"S/51°29'15"W) and Southeast (24°42'29"S/47°3319"W). A total of 898 birds were captured during 2005 and 2006; blood samples were collected from all of them and cloacae swabs were collected from 307 wild birds. Most of the analyzed birds belonged to the order Passeriformes (85.3%; 766/898) considered an important viral reservoir. The attempt for virus isolation from swab samples was performed on Vero E-6 and C6/36 and by i.c. inoculation in suckling mice. Culture supernatants and swab samples were also analyzed by RT-PCR for viral RNA detection. Blood samples were tested for antibodies by Hemagglutination Inhibition (HI) assay. In none of the analyzed samples either WNV or other arbovirus was isolated as well the RT-PCR results were also negative in the tested samples. HI results indicated the presence of antibodies against Iguape virus (IGPV), Saint Louis virus (SLEV), and Ilhéus virus (ILHV) with predominance of monotypic reactions. In two samples, heterotypic reaction for WNV was detected; however this result is insufficient to confirm the introduction of the virus in Brazil. Taken together, the results of this study prove that, at least up to 2006, WNV had not been introduced into São Paulo state, and probably into Brazil.

## 1167

### CO-CIRCULATION OF DIFFERENT DOBRAVA HANTAVIRUS LINEAGES IN A*PODEMUS* MICE IN SLOVENIA

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Hantaviruses are associated with their natural reservoir hosts, either rodents or insectivores. The chronically infected animals excrete the virus in urine, feces and saliva, without developing a disease. Hantaviruses are usually closely associated with one rodent or insectivore species as a result of a co-evolution of the virus and the host. However, deep phylogenetic analyses have shown inconsistence in the pattern of host and virus co-evolution: besides to host switching events, numerous hantaviruses have been reported to be isolated from different sympatric hosts. Such spillover infections are quite common and promoted with complex bio-geographic and anthropogenic pressures on the environment, but their impact on the public health is still undetermined. A severe form of hemorrhagic fever with renal syndrome in Europe is caused by Dobrava hantavirus, carried by *Apodemus flavicollis*. In addition to *A. flavicollis* several other *Apodemus* mice have been shown to carry DOBV-like hantaviruses. In light of monitoring the hantavirus spread in Slovenia in natural environment, the rodent trapping is conducted on several locations twice a year. Using Sherman type live traps several rodent species were caught from 1990 to 2009 and three *Apodemus* mice species were selected: a yellow necked mouse (*A. flavicollis*), a striped field mouse (*A. agrarius*) and a long-tailed mouse (*A. sylvaticus*) for detailed inspection. Both, serologically and molecularly in all three species hantaviral infections were identified. Above that, all *Apodemus* species were positive in several years and on different locations, implying that this is not only a spillover infection as an effect of favouring environmental conditions. Molecular analyses proposes that different DOBV-like hantaviruses circulate in natural reservoir in Slovenia, but only the Dobrava virus prototype, isolated from yellow-necked mouse, is undoubtedly causing a disease in humans.

## 1168

### MONKEYPOX PATHOGENESIS STUDY USING A SERIAL SACRIFICE TECHNIQUE

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Previously, we have shown the prairie dog is a valuable model system which closely mimics human systemic orthopoxvirus disease. To further characterize the strains of monkeypox virus (MPXV) and increase our understanding of MPXV progression within an animal host, we challenged groups of prairie dogs with two MPXV strains, one Congo Basin origin and one West African origin (each at 8 X $10^3$ PFU), and evaluated disease progression in tissues on days 2, 4, 6, 9, 12, 17 and 24. Viral loads in 28 different tissues from animals were evaluated and compared. Animals challenged with Congo Basin strain had virus recovered on day 4 from nasal cavity, spleen, and submandibular lymph node tissues; earlier than West African challenged animals (day 6). For both MPXV strains, the majority of tissues were positive for virus between days 6-9. Tissues later infected by virus (day 12) included gallbladder, lesion and blood (both strains) and additionally for the West African strain on day 12; brain, heart, mesentery lymph nodes, pancreas, stomach and urine/bladder tissue. Two animals that succumbed to disease on day 12, when evaluated by IHC and histopathology, demonstrated abundant viral antigen in all organs with the exception of the brain. These findings allow for the better understanding of the pathogenesis of MPXV, including identification of sites important during early replication of the virus. This data could

prove useful in the development of therapeutic and biologic agents and in understanding the disease differences observed between the MPXV clades.

## 1169

### SEROPREVALENCE OF ANTIBODIES AGAINST ARBOVIRUSES IN MBEYA REGION, SOUTHWESTERN TANZANIA

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Arboviruses cause some of the most important human infections. Although several arbovirus epidemics have originated in East Africa over the last years, few recent data are available on the endemic prevalence of arboviruses in this area, and no data is available from South-West Tanzania. In the current study 1.233 sera were tested that had been collected from participants of the EMINI study in the Mbeya region in South-West Tanzania. Nine sampling sites were selected to cover altitudes from 500 m to 2.300 m above sea level (asl) and different ecologic conditions. Sera were tested for antibodies against Rift Valley Fever virus (RVFV), yellow fever virus (YFV), West Nile virus (WNV), Chikungunya virus (CHIKV), Dengue -1 to -4 viruses (DENV-1 to DENV-4), using an immuno-fluorescence based biochip designed for this study (Fa. Euroimmun, Lübeck, Germany). In two of the nine sites, namely Igurusi (1.100 m asl; east of Mbeya city) and Kyela (500 m asl; on the shores of Lake Malawi), high antibody prevalences against all tested arboviruses were detected. In Kyela anti-CHIKV prevalence was 49%, anti-RVFV was 24%, and anti-flavivirus prevalences ranged from 16 to 27% with higher titers against WNV and DENV-3. The other sites, which were located at mean elevations between 1.300 and 2.300 m asl, showed IgG seroprevalence rates between 0 to 13%. Additional testing of IgM in Kyela site showed preliminary IgM antibody rates of up to 10% against the above arboviruses. Our data demonstrate a non-epidemic circulation of several arboviruses, including CHIKV, WNV, DENV-3 and RVFV, in Mbeya region in South-West Tanzania, none of which had previously been diagnosed in the region. As antibodies against the tested arboviruses were mainly found in areas below 1.100 m, we assume that these arboviruses circulate in regions below 1.100 m in an endemic pattern. The arboviruses circulating should be characterized in detail as they may pose a virus reservoir for future epidemics and pandemics.

## 1170

### SEROLOGIC EVIDENCE FOR MULTIPLE GENERA OF POXVIRUSES CIRCULATING IN THE PERIDOMESTIC RODENT POPULATION IN WESTERN UGANDA

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Rodents are implicated as the likely reservoir host for many zoonotic poxviruses. Little is known concerning the prevalence of poxvirus infections among specific rodent species in the wild. Our current study investigated poxvirus exposure among peridomestic rodents in western Uganda. We identified antibodies to two separate genera of the family *Poxviridae*. Western blot and ELISA studies of rodent serum identified a reactive antibody response to viral proteins found in the genera *Orthopoxvirus* and *Yatapoxvirus*. This implies rodents in western Uganda are exposed to at least two poxviruses. The previous poxvirus exposures in these rodents may be due to known zoonotic human pathogens (such as cowpox, monkeypox, or tanapox virus) and/or currently unknown, novel poxviruses. Endemic zoonotic poxviruses are not known to be circulating in the human population in western Uganda. The current study provides serologic evidence of poxvirus exposure among peridomestic rodents which could represent a concern for human health.

## 1171

### IDENTIFICATION OF A NOVEL ANTIVIRAL DRUG AGAINST BLUETONGUE VIRUS

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Bluetongue virus (BTV), the prototype virus in the genus *Orbivirus* within the *Reoviridae* family, is transmitted to its vertebrate host by certain species of *Culicoides* biting midges. BTV disease is one of the most important diseases of domestic livestock, causing $3 billion/year loss worldwide. While BTV vaccine is available and has been used to prevent BTV diseases, the recent outbreak of BTV in northern Europe indicates a pressing need for antivirals to fight against this disease. We present the identification and characterization of a novel antiviral against BTV. This novel antiviral small molecule compound belongs to one of the six cluster if antivirals against BTV, as reported previously, identified via a high throughput screening of a 200,000 compound library. This compound showed an IC50 at 0.69 ± 0.13 μM, with very low cytotoxicity (CC50 >50 μM), demonstrated that it is high selective against BTV with a Selective Index (SI50) over 50. This compound also reduced the BTV plaque formation by 2-3 logs in standard plaque assay. The Time-of-Addition assay showed that this compound inhibited the early event of the BTV viral life-cycle. Mechanism of action studies indicated that it might interact with the BTV viral replication machinery. The above results demonstrated that the novel antiviral against BTV could be identified and characterized for future drug discovery and development to prevent BTV diseases.

## 1172

### ARBOVIRUS STRAIN PHENOTYPES CAN BE CHARACTERIZED VIA DYNAMIC ESTIMATES OF VECTORIAL CAPACITY AND THE DISPLACEMENT INDEX

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Vectorial capacity (VC) is defined as the number of infections that a specific mosquito population can distribute per case per day, and is used as a measure of the transmission potential of a vector borne pathogen into a susceptible population. Since its original description by McDonald-Ross, an important modification has been to include a transmission capability parameter, vector competence. Vector competence is the intrinsic ability of an arthropod to transmit an infectious agent following exposure to that agent. While traditional comparisons of vector competence are informative, the best method for comparing the transmission potentials of arboviruses uses the rate of change of vector competence over an interval of time meant to represent a range of extrinsic incubation periods. Using published data, we demonstrate the validity of this VC model when comparing intrinsic viral characteristics as related to fitness phenotypes both within a single mosquito species as well as among two species. Our results demonstrate, through calculation of the Displacement Index (DI), that the relative fitness of the West Nile Virus strain that was first introduced into the United States (NY99) was inferior to an emergent strain identified in 2002 (WN02). The DI shows, relative to an established strain of virus, the potential of another strain to overtake and displace that established strain in the system. For example, the DI of WN02 to NY99 is 2.14, indicating that WN02 has a significant fitness advantage for transmission than does NY99. The culmination of this displacement was seen as WN02 spread across the US. We also analyzed the inter and intra-specific relationship of a chikungunya virus (CHIKV) strain before and after a valine substitution that gave rise to the epidemic strain of the 2006 outbreak on La Reunion and Southern India. Comparisons of CHIKV phenotypes were made within and between *Aedes*

*aegypti* and *Ae. albopictus* mosquitoes. The cumulative VC and DI allow for measures of viral strain differences and relative fitness estimates within a dynamic transmission system.

## 1173

### PRIME-BOOST MALARIA VACCINES IN RHESUS MONKEYS USING PROTEIN IN POLY I:C ADJUVANT AND ADENOVIRUS VECTORS

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Development of an effective vaccine against malaria is an urgent priority in global public health. Vaccines directed at the liver stage of malaria infection work by inducing both antibody and cellular immune responses. Recombinant protein-based vaccines are strong inducers of antibody responses, while recombinant attenuated adenovirus (Ad) vaccines are strong inducers of T cell immune responses. Both have protected mice against malaria alone and after priming animals with other vaccine constructs. In this study, we evaluated the immunogenicity and protective efficacy of recombinant protein and Ad vaccines using the *P. knowlesi* (Pk)-*M. mulatta* model. The Pk circumsporozoite protein (CSP) was used in both the protein and adenovirus constructs. All protein vaccinations included Poly I:C (PIC) adjuvant. The following vaccine strategies were compared: protein prime/Ad5 boost; Ad5 prime/protein boost; Ad28 prime/Ad5 boost; and protein prime/protein boost. Control groups received Ad not encoding malaria antigen and PIC adjuvant without protein. Four weeks after the final vaccination, all monkeys were challenged with Pk sporozoites by mosquito bite and followed for 30 days for the development of parasitemia by thin and thick blood smears. Blood from all monkeys was collected throughout the study and antibody and T cell responses to the CSP were analyzed. We will present the immunogenicity and parasitemia data from the study, along with correlations between immune responses and protective efficacy.

## 1174

### EVALUATION OF IMMUNE RESPONSES TO A *PLASMODIUM VIVAX* CSP-BASED RECOMBINANT PROTEIN VACCINE CANDIDATE IN COMBINATION WITH SECOND-GENERATION ADJUVANTS IN MICE

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*Plasmodium vivax* is the major cause of malaria outside of sub-Saharan Africa and inflicts debilitating morbidity and consequent economic impact in developing countries. In order to produce a *P. vivax* vaccine for global use, we have previously reported the development of VMP001, based on the circumsporozoite protein (CSP) of *P. vivax*. VMP001 is a novel recombinant protein encompassing the N-terminal and C-terminal regions flanking a chimeric repeat region representing VK210 and VK247, the two major alleles of *P. vivax* CSP. Our interest is to evaluate second-generation vaccine formulations to identify novel combinations of adjuvants capable of inducing strong, long-lasting immune responses. In this study groups of C57BL/6 mice were immunized subcutaneously three times with VMP001 in combination with synthetic TLR4 (GLA) or TLR7 agonist (R848) in stable emulsion (SE), or SE alone. Sera and splenocytes were tested for the presence of antigen-specific humoral and cellular responses, respectively. All groups of mice generated high titers of anti-*P. vivax* IgG antibodies as detected by ELISA and immunofluorescence assay. GLA-SE promoted a shift in the antibody response to a Th1 profile, as demonstrated by the IgG2c/IgG1 ratio. In addition, GLA-SE induced a strong cellular immune response characterized by multi-functional, antigen-specific CD4+ T cells secreting IL-2, TNF and IFN-γ. In contrast, mice immunized with SE or R848-SE produced low numbers of antigen-specific CD4+ T cells and these T cells secreted both IL-2 and TNF, but not IFN-γ. Finally, R848-SE did not enhance the immune response to GLA-SE alone. We conclude that the combination of VMP001 and GLA-SE is highly immunogenic in mice and may serve as a potential candidate second-generation vaccine against vivax malaria.

## 1175

### POLYPEPTIDE NANOPARTICLES GENERATE CD8⁺ T-CELL RESPONSES IN THE ABSENCE OF ADJUVANT

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Protection against malaria is considered to rely mostly on antibodies to block or destroy sporozoite and blood stage parasites and primarily on CD8+ T-cells for destruction of the liver stage of infection. A single highly effective malaria vaccine has not yet been formulated that induces both antibody and cellular immune responses. In order to generate CD8+ T-cell responses exogenously derived proteins need to be cross-presented, as the normal processing of exogenous protein will generate mainly CD4+ T-cell responses. It is a common belief that cross-presentation of antigen usually requires either the use of viral vectors or strong adjuvants. We recently described a platform of a Self-Assembling Polypeptide Nanoparticle (SAPN) capable of generating protective antibodies against the CS repeat of the CSP from *P. berghei*. We now report that the same formulation is able to deliver the *P. berghei CSP* CD8+ T-cell epitope SYIPSAEKI to induce an effective cellular immune response. Mice were immunized with 10μg SAPN (Pb2) bearing the peptide SYIPSAEKI 3 times, 2 weeks apart. Two weeks post last immunization, animals were sacrificed and splenocytes harvested from spleens to analyze for Ag-specific CD8+ T-cell activation *in vitro* by ELISpot and ICS analysis. Splenocytes from immunized animals responded positively to *in vitro* stimulation with 10μg/ml of SYIPSAEKI by secreting IFNγ (0.05 % in immunized animals versus 0.005 % in the control). Exposure to SYIPSAEKI also induced significant production of IL-6, Rantes and MIP. These results indicate that SAPN can deliver a CD8+ T-cell epitope to induce antigen specific cellular immune responses. This opens the door to the design of a single nanoparticle that can be delivered without adjuvant to induce a protective antibody response that can be coupled with an effective cellular response. We believe that we can now design and delivery a truly effective single component malaria vaccine.

## 1176

### QUANTIFYING INVASION INHIBITORY ANTIBODIES TO AMA1 IN HUMAN POPULATIONS USING TRANSGENIC *PLASMODIUM FALCIPARUM*: IMPLICATIONS FOR VACCINE DESIGN

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Antibodies to merozoite antigens are an important component of acquired immunity to *P. falciparum* and act in part by inhibiting erythrocyte invasion. However, the major targets of protective and invasion-inhibitory antibodies remain unclear. Apical membrane antigen 1 (AMA1) is an essential erythrocyte invasion ligand and leading vaccine candidate. Antibodies raised against AMA1 inhibit the growth of blood stage parasites, but are constrained by strain-specificity. A successful AMA1 vaccine may require the inclusion of multiple AMA1 alleles to overcome this limitation. To quantify the importance of AMA1 as a target of acquired inhibitory antibodies in humans and understand the acquisition of antibodies to different AMA1 alleles, we have successfully generated transgenic *P. falciparum* lines expressing different AMA1 alleles on the same genetic background. Using these parasites in invasion inhibition assays enables the measurement of AMA1-specific inhibitory antibodies distinct from other antigen-specific antibodies. Testing antibodies among a cohort of children and adults in Papua New Guinea revealed that AMA1 is a major target of acquired invasion inhibitory antibodies. Acquired inhibitory antibodies showed substantial strain-specificity, and the prevalence of inhibitory antibodies differed significantly for different AMA1 alleles, suggesting different rates of acquisition of antibodies to different alleles. These results have important implications for vaccine development and understanding the targets of protective and inhibitory antibodies in humans. Transgenic parasites may be valuable for measuring antibody specificity and functional activity in AMA1 vaccine trials.

## 1177

### QUANTIFYING THE IMPORTANCE OF PFEMP1 AND OTHER ANTIGENS EXPRESSED ON THE SURFACE OF *PLASMODIUM FALCIPARUM*-INFECTED ERYTHROCYTES AS TARGETS OF PROTECTIVE ANTIBODIES AGAINST MALARIA

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*Plasmodium falciparum*-infected erythrocytes (IEs) express variant surface antigens (VSAs) that are major targets of immune responses. Antibodies to VSAs develop in a largely variant-specific manner and are associated with protection from symptomatic and severe malaria. Several confirmed or proposed VSAs have been identified, including PfEMP1, RIFIN, STEVOR, and SURFIN protein families, and others. However, the relative importance of these different proteins as targets of acquired antibodies remains unclear. To address this, we have used parasite lines in which surface expression of PfEMP1 and other antigens was inhibited by transfection of parasites with specific constructs that either i) suppress var gene expression or ii) interfere with trafficking of antigens to the IE surface. We developed novel assays to measure antibodies to PfEMP1 and other VSAs on the surface of IEs by comparing antibody reactivity to transfected versus parental parasites. Using this approach with samples from Kenyan children and adults we have quantified the importance of PfEMP1 and other VSAs as targets of acquired antibodies and we have related these antigen-specific responses to protective immunity in cohort studies. Furthermore, to understand the functional relevance of antibodies to different VSAs, we have measured antibody opsonic phagocytosis activity using parasites with modified VSA expression compared to parental parasites. These studies have enabled us to quantify the importance of different VSAs as targets of acquired antibodies, and their likely role in protective immunity to malaria. These findings have significant implications for understanding human immunity to malaria and informing vaccine development.

## 1178

### DIFFERENCES IN HUMORAL IMMUNITY AGAINST *PLASMODIUM FALCIPARUM* MALARIA IN MALIAN CHILDREN CARRYING NORMAL HEMOGLOBIN A OR SICKLE HEMOGLOBIN S

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Previous studies have shown that children with sickle-cell trait (HbS heterozygosity) have reduced incidence of *Plasmodium falciparum* malaria compared to normal (HbA homozygosity) children. While we still do not understand the mechanisms responsible for this protection, enhanced immunity by sickle-cell trait has been proposed to play a role. To develop a more comprehensive picture of anti-malarial immunity in these African children, we initiated a 5-year longitudinal cohort study of 73 HbS and 126 HbA children in Mali. As part of this study, we followed these children through a 6-month malaria transmission season, collecting plasma from all children at the beginning (May 2009) and end (December 2009) of the transmission season to compare their immune responses to *P. falciparum* antigens. As expected, HbS children experienced significantly fewer malaria episodes than HbA children during this period. To compare the development of humoral immune responses between the two groups of children, we used a standardized ELISA to quantify antibody titers against 4 erythrocytic-stage antigens (AMA1, MSP1, EBA175, and MSP2). Among the children aged 6-11 years, HbS children showed significantly lower antibody titers to several of the antigens in May compared to HbA children. While these titers increased in both groups during the transmission season, similar differences in titers were found in December (HbS<HbA). We hypothesize that the lower antibody titers in the HbS children were due to fewer malaria episodes and consequently less exposure to parasite antigens. However, there was no correlation between the number of malaria episodes during the transmission season and the increase in ELISA titers in both HbS and HbA children. Therefore, other mechanisms are likely involved in modulating levels of *P. falciparum*-specific antibodies in HbS children. Additional immunological measures (e.g., functional activity of anti-malarial antibodies, etc) will be performed to compare the HbA and HbS populations.

## 1179

### DECLINING BLOOD-STAGE IMMUNITY IN THE SETTING OF DECREASING MALARIA INCIDENCE IN UGANDA

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In endemic areas, protective immunity against *Plasmodium falciparum* usually increases with age and cumulative exposure. However, successful

malaria control efforts are leading to decreased intensity of parasite exposure in many areas. The consequences of decreased exposure on antimalarial immunity are unclear. We recently suggested that an increasing risk of treatment failure in response to amodiaquine + sulfadoxine-pyrimethamine in a closely managed cohort of children in Kampala, Uganda, was due to declining immunity and not increased drug resistance, prompting us to investigate other manifestations of blood stage immunity. 601 randomly selected children from Kampala, aged 1-10, were followed for a median of 1.4 years. Blood smears were read every 30 days and any time a child presented with fever. Children with malaria, defined as asexual parasites on blood smear and fever, were treated after randomization to one of 3 combination therapy regimens. To follow parasite strains within individuals over time, parasitemic samples were genotyped by assessment of polymorphisms in *merozoite surface protein 2* by nested PCR and capillary electrophoresis. We estimated associations between calendar time and two measures of immunity, the ability to avoid clinical illness despite parasitemia and the ability to spontaneously clear a parasite strain without receiving therapy, in both cases adjusting for age and accounting for repeated measures within individuals. During the study the incidence of malaria fell from 1.6 to 0.9 episodes per person year and 375 children (62%) had at least one positive blood smear. The probability of avoiding symptoms despite parasitemia (OR=0.38 per year, 95%CI=0.26-0.56, p<0.001) and the probability of spontaneously clearing an infection (OR=0.29 per year, 95%CI=0.13-0.64, p<0.01) decreased significantly over time. These data suggest that clinically relevant and readily measurable blood stage immunity declined over a short period of time in our cohort, possibly due to improved access to effective therapy and decreasing parasite transmission intensity.

## 1180

### A GENOME WIDE TRANSCRIPTIONAL STUDY INVESTIGATING CANDIDATE GENES IMPORTANT FOR DESICCATION RESISTANCE IN *ANOPHELES GAMBIAE*

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*Anopheles gambiae* plays an important role in malaria transmission. In Africa, the dry season can last 2-3 months and malaria vector come back right after onset of the rainy season. How they survive through the dry season is still poorly understood. It is plausible that *An. gambiae* may have increased tolerance over the dry season while in the egg or larvae stages. Genetic analysis has shown that mosquitoes carrying inversion distribution on 2La and 2Rs (2Rb, 2Rc, 2Rd and 2Rd) are non-randomly correlated to aridity; therefore the genes involved within these inversions are worth to explore desiccation resistance. A genome-wide study of *An. gambiae* can identify transcript profiles of genes and pathways involved in desiccation resistance, shedding light on the possible survival strategies of malaria vectors during the dry season.

## 1181

### INFLUENCE OF 2L*A* CHROMOSOMAL INVERSION ON DESICCATION RESISTANCE OF *ANOPHELES GAMBIAE S.S.* FROM CAMEROON

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Many natural populations show geographic clines in traits including gene frequencies, morphological characters, and physiological or behavioral responses that can be interpreted as adaptive responses resulting from contrasting selective regimes. The frequency of the 2L*a* chromosomal inversion in *Anopheles gambiae sensu stricto* is associated across Africa with the degree of aridity, resulting in Cameroon in the clinal distribution of carriers of alternative karyotypes for this inversion along a latitudinal gradient: homokaryotypic standard arrangements prevail in the southern humid rainforest, whereas homokarytotypic inverted arrangements predominate in the northern dry savanna. Accordingly, this inversion is believed to capture genes that could be involved with adaptation to more arid conditions. The physiological bases underlying such adaptation, however, are as yet unknown. To study the relationship between the 2L*a* inversion and resistance to desiccation, we subjected the F10-F12 of an *Anopheles gambiae s.s.* molecular form S strain originating from a polymorphic population from Eastern Cameroon to a desiccation stress-test. Newly emerged male and female mosquitoes were put in sealed glass vials containing a desiccant and their survival was followed with an automated video-control system until death. Using a molecular diagnostics for this inversion, we were able to measure differences in survival of alternative 2L*a* karyotypes controlling for confounding covariables such as sex and body size during the test. Homokaryotypic inverted female mosquitoes survived, on average, significantly longer in a desiccated environment than females of the other karyotypes (+120 min), and than males, whatever their karyotype (+180 min).

## 1182

### MOLECULAR EVOLUTION OF GENES INVOLVED IN POST-MATING REPRODUCTIVE MECHANISMS OF THE MALARIA MOSQUITO *ANOPHELES GAMBIAE*

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Proteins involved in reproduction evolve rapidly due to positive selection resulting from intersexual interaction and sexual conflict. In *Drosophila*, rapid evolution driven by positive selection has been detected in proteins expressed either in the male accessory glands (MAGs) and in the female lower reproductive tract (LRT). In *Anopheles gambiae* MAG-products are transferred to females as a solid mating plug that induces a series of physiological post-mating responses in females. We here report data on the molecular evolution in five members of the *A. gambiae* complex (*A. gambiae* s.s., *A. arabiensis, A. quadriannulatus, A. melas and A. merus*) of two clusters of 3 LRT- and 3 MAG-specific genes potentially involved in post-mating mechanisms. The 3 LRT-specific genes encode serine-proteases that are down-regulated after mating, two of which are expressed in the atrium (and interact with the mating plug) and one in the spermatheca. Adaptive evolution was detected in several codons of the 3 genes; moreover, episodic selection was inferred in the spermatheca-specific gene along the branch leading to *A. melas*. The particularly high level of replacement polymorphisms in all 3 proteases suggests that, as in *Drosophila*, these duplicated genes might experience relaxed evolutionary constraints that could be important to rapidly explore and eventually fix new advantageous variants. Among the 3 MAG-specific paralog genes, two are conserved, whereas one is highly differentiated among *A. melas*, *A. merus* and *A. quadriannulatus*, due to positive selective pressure and purifying selection maintaining lineage-specific products.Overall, the evolution of these genes appears to be consistent with a model of sexual conflict, in line with their crucial role in *A. gambiae* reproduction. The association of evolutionary and functional analyses might help clarifying their role in post-mating responses and, possibly, in maintaining reproductive isolation among *A. gambiae* species, thus hopefully providing new targets for the development of novel malaria vector control strategies.

## 1183

### IDENTIFYING THE *ANOPHELES GAMBIAE* PROTEIN KINASE C GENE FAMILY AND EXPLORING ITS ROLE IN MOSQUITO INNATE IMMUNITY

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With nearly 40 percent of the world's population at risk for malaria infection, it is necessary to explore novel strategies of malaria control. One approach to decrease disease transmission is to target and alter immune genes in the mosquito to reduce the parasite burden and disable vector competence.

The highly conserved protein kinase C (PKC) gene family has been shown to regulate wide-ranging immune functions in a variety of vertebrate and invertebrate species. Based on these observations, we sought to identify and characterize all of the PKC isoforms encoded within the genome of the African malaria mosquito Anopheles gambiae as a prelude to functional studies. A total of 13 PKC isoforms are known from mammals and 6 are known from Drosophila melanogaster. However, prior to our studies, only two PKC-encoding genes had been identified in the genome of A. gambiae. Using Hidden Markov Model (HMM) searches of the translated reading frames of the unannotated A. gambiae genome sequence, we confirmed the identity of the two previously annotated PKC-encoding genes, identified an additional three PKC-encoding genes and a gene encoding a PKC-related kinase 2 (PKN2) ortholog. Subsequently, we identified conserved domains, putative translational start sites, and phosphorylation sites required for catalytic function of the predicted proteins using ClustalX and manual alignments of transcriptionally validated orthologous sequences. Expression data for of all but one of these PKC-encoding genes have been deposited in publically available databases. Phylogenetic analyses were performed using PAUP* 4.0 and PHYLIP, revealing close relationships between the newly identified A. gambiae PKCs with other dipteran PKCs within the same subfamily. It has been shown that insulin signaling can activate PKCs via phosphorylation or cellular translocation in vertebrates. In immortalized A. gambiae cells lines Sua5B and 4a3B, phosphorylated PKCs mu and zeta are upregulated in response to insulin treatment. Interestingly, phospho-PKC mu is translocated both to the cell membrane and to the nucleus in response to insulin treatment. With this new knowledge of the PKC gene family in A. gambiae, we can continue to characterize the functions of these proteins in host physiology.

## 1184

### MOSQUITO IMMUNE CELLS FORM SESSILE FOCI IN RESPONSE TO INFECTION

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Mosquitoes counter the threat of microbial infection with a capable innate immune response that relies heavily on the action of immune cells (hemocytes) that are found circulating with the hemolymph or attached to tissues. Changes in sessile hemocyte populations following immune challenge have been reported in other insect taxa, but remain poorly understood and have not been studied in mosquitoes. We have developed an effective method of staining mosquito hemocytes *in vivo* along with a means of consistently perfusing approximately 95% of circulating hemocytes from an adult mosquito and have used these techniques to study the effect of immune challenge on the numbers of sessile and circulating hemocytes in *Anopheles gambiae*. Qualitative studies showed that sessile hemocytes occur throughout the mosquito's body but tend to concentrate in specific regions and increase in abundance following immune challenge. These studies also revealed the identity of previously reported phagocytic foci near the abdominal ostia as large aggregates of hemocytes and showed that formation of these foci can be induced by inoculation with bacteria or inert particles. Lysosomal staining confirms that the foci are engaged in degradation of the phagocytosed materials and in some cases the formation of foci is coupled with a melanization response. Quantitative analyses showed that the number of hemocytes in phagocytic foci increases in a dose dependent manner following immune challenge and that foci form on a consistent time scale. Analyses of the systemic immune response showed that total hemocyte numbers increase significantly following immune challenge and that there is a significant increase in the number of circulating hemocyte aggregates following immune challenge. Together, these data demonstrate a novel cellular immune response in mosquitoes.

## 1185

### WEST NILE VIRUS-BINDING PROTEINS IN THE MIDGUT OF *CULEX PIPIENS QUINQUEFASCIATUS* SAY AND *C. NIGRIPALPUS* THEOBALD (DIPTERA: CULICIDAE)

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It is known that different species and populations of mosquitoes show differential vector competence, however, the molecular mechanisms that contribute to vector competence variation at the level of the mosquito midgut are largely unknown. Midgut virus-binding proteins may represent a mechanism that contributes to vector competence. We examined virus-binding midgut proteins in two important West Nile virus (WNV) vectors, *Culex pipiens quinquefasciatus* and *Cx. nigripalpus*. Polyacrylamide gel electrophoresis showed that there were at least 15 midgut proteins in *Cx. p. quinquefasciatus*, ten of which bound WNV after a virus overlay binding assay. The proteins that bound WNV ranged in size from 38 kDa to 198 kDa. Polyacrylamide gel electrophoresis of *Cx. nigripalpus* midgut proteins revealed that there were at least 21 midgut proteins, seven of which bound WNV after a virus overlay binding assay. The *Cx. nigripalpus* midgut proteins that bound WNV also ranged in size from 38 kDa to 198 kDa. These results are consistent with midgut virus-binding proteins from *Aedes aegypti* that bind dengue virus, including one, a 67 kDa protein, that has been related to vector competence and may serve as a genetic marker. We provide a protein expression profile from two different vectors of WNV. The involvement of the WNV-binding proteins in virus entry into mosquito midgut epithelial cells will be discussed.

## 1186

### A REGULATORY UNIT OF THE MELANIZATION RESPONSE AFFECTS THE LIFE SPAN OF MOSQUITOES

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Melanization is a powerful innate immune response in the arthropods that leads to encapsulation and killing of invading pathogens. This process renders some mosquito species partially or completely resistant to infection with pathogens of global public health significance. However, if not properly controlled, melanization reduces the life span of the mosquito itself. The rate-limiting step in the process of melanogenesis is the activation of prophenoloxidase (PPO), which is controlled by an extracellular protease cascade and associated serpin inhibitors with largely unknown molecular composition in mosquitoes. A notable exception is *Anopheles gambiae* serpin (SRPN)2 and its orthologs in other mosquito species, which were previously identified as key negative regulators of melanization. The aim of this study was to identify the molecular target of SRPN2 in *An. gambiae* and thus identify a regulatory unit of the PPO activation cascade in mosquitoes. Using a combination of reverse genetic and biochemical techniques we identified the *An. gambiae* clip-serine protease CLIPB9 as a PPO-activating protease (PAP). Double-knockdown of SRPN2 and CLIPB9 significantly reversed the pleiotrophic phenotype induced by silencing of SRPN2, including rescue of melanotic tumor formation and shortened life span. Recombinant activated CLIPB9 forms

SDS-stable complexes with SRPN2 *in vitro* and in mosquito hemolymph, which leads to the inactivation of the protease. The association rate constant of this complex and the stoichiometry of inhibition are comparable to known inhibitory serpin-protease interactions. Furthermore, recombinant CLIPB9 cleaved and activated purified insect PPO. This study identifies the first inhibitory serpin-serine protease pair in mosquitoes, thereby defining a regulatory unit of the biochemical cascade that is essential for melanization in the mosquito innate immune response. To the best of our knowledge, CLIPB9 is the first bona fide PAP to be described in a dipteran species, including *Drosophila melanogaster*.

## 1187

### PAN PHYLUM ANALYSIS OF PROTEIN-PROTEIN INTERACTIONS REVEALS POTENTIAL DRUG TARGETS FOR HELMINTHES

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Parasitic helminths have deleterious effects on human health, livestock, and plants, costing society billions of dollars annually. Finding new drug targets for parasitic infections would be of great utility for humanity, as there is a large need to develop new drugs to fight parasitic helminth infections due to the developing resistance and side effects of current treatments. This study underlines three major principles: i) proteins that are essential and conserved among species that span a phyla are of greatest value, as they provide foundations for developing broad control strategies, ii) Parasite proteins that share homology to the host counterpart are also of a great value when they posses molecular features that are unique to the parasite therefore are candidates for selective targeting, and iii) proteins rarely act in isolation, and the majority of biological processes occur via interactions with other proteins, so protein-protein interactions offer a realm of unexplored potential drug targets. Here we present a computational approach which which builds on these three principles, utilizing complete proteomes of the model free-living *Caenorhabditis elegans*, 6 parasitic helminthes and 2 of their hosts. Markov clustering of the proteins resulted in orthologous families that could be placed in species specific groups. Protein-protein interactions within these species specific groups were identified by comparisons to evidence based protein-protein interactions. Protein-protein interactions specific to nematodes were prioritized and scored based on RNAi phenotype and homology to the PDB. In addition, investigation of the parasite protein-protein interactions shared with the host resulted in amino acid insertions and deletions specific to the parasites. Developmental gene expression profiles, functional annotation (GO), and druggability were also considered. Several protein-protein interactions unique to nematodes or with nematode specific amino acid insertions and deletions emerged from this study and provide novel potential drug targets for controlling parasitic helminth infections.

## 1188

### THREE NOVEL GUINEA WORM *(DRACUNCULUS MEDINENSIS)* GENOMIC SEQUENCES

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*Dracunculus medinensis* is a parasitic nematode that causes dracunculiasis (Guinea worm disease). In 1986, the World Health Assembly resolved to eradicate dracunculiasis. Sequencing of the *D. medinensis* genome before complete eradication of dracunculiasis and the parasite is a high priority project. The only available genomic sequences of *D. medinensis* are for the 18S ribosomal RNA multicopy gene determined by Bimi et al., 2005 and Wijova et al., 2006. Additional data obtained for single-copy genes will be useful for verification of *D. medinensis* genomic sequencing results. We aligned the available genomic sequences for the nematode order Spirurida to identify conserved regions within the heat shock protein 70 (HSP70) gene and to use them to design PCR primers. Using these primers under low stringency conditions, we amplified and directly sequenced three PCR products of 517, 1952 and 2584 bp. The shortest and mid-size products were found to represent a portion of the utrophin exon with an adjacent intron and three exons flanked with introns of the hammerhead gene, respectively. Their sequences were submitted to GenBank under accession numbers HM131215 and HM131214. The longest product showed 70% similarity with the *Wuchereria bancrofti* HSP70 gene. Starting from that partial HSP70 sequence, we extended it to full length by DNA walking and submitted sequence data to GenBank (HM125969). The alignment of this full length sequence with several Spirurida HSP70 gene sequences identified 11 coding exons including exons 4A and 4B that were separated by an intron of 73 bp. A similar intron was not detected in orthologous HSP70 genes of other Spirurida studied to date; all of them had a single exon 4. The *D. medinensis* HSP70 amino acid sequence showed over 95% similarity to available Nematoda HSP70 protein sequences and phylogenetic analysis revealed significant divergence between *D. medinensis* and other Spirurida HSP70 gene sequences.

## 1189

### UNRAVELING THE BIOLOGY OF AUTOINFECTION BY *STRONGYLOIDES STERCORALIS*: A MICROARRAY BASED ANALYSIS

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An unusual feature of the life cycle of Strongyloides stercoralis (Ss) is its ability to persist for years in infected hosts, by the process termed autoinfection. During autoinfection, larvae develop precociously within the host of origin to the third stage (L3a), penetrate the colonic wall/perianal skin and migrate via a variety of routes including the lungs. The molecular differences between autoinfective larvae and infective larvae which develop outside the host (L3i) have been uncharacterized to date. We conducted a microarray analysis to compare gene expression of L3a and L3i larvae recovered from experimentally infected animals. Differentially labeled cDNA obtained from RNA extracted from larvae were hybridized to a Ss microarray. Genes that were more highly expressed in either stage (based on a conservative cutoff of 2 fold increased gene expression and microarray signals with $p < 0.01$; false discovery rate of 1%) were examined for differences in gene function based on a novel cDNA annotation system. In a preliminary analysis, 600 of 3571 genes on the array were identified as being differentially expressed. Striking differences in gene expression were found between the two stages with higher numbers of L3a genes involved in transcription (p=0.03), molecular chaperones (p=0.03), signal transduction (p=0.01), vesicular transport (p=0.03) and metabolism (p=0.0002). L3a upregulation of the ubiquitin proteasome system may be critical to Ss larval development and differentiation in the host. In addition, a potential therapeutic target, a highly expressed and abundant L3a nucleoside hydrolase (Ss-contig 2570), was identified. Upregulation of L3i cuticular collagens (p=0.004) likely enable survival in harsh environmental conditions. Increased numbers of L3i ferritin transporters implicates a role for iron metabolism in the response to environmental stress. These data provide valuable insights into how Ss larvae adapt to stress induced by the environment and host immune system that can then be applied to the development of novel therapeutic and vaccine targets.