or frank arthritis (66.6%), hepatomegaly and/or splenomegaly (61.1%). The most common laboratory finding was elevated transaminases (ALT [median (range)]: 74 (38-616); AST: 78 (27-782) IU/L). Three adults (50%) but no children had thrombocytopenia (platelets <100,000/mcL, *P=0.025*). In conclusion, in the Southern U.S., brucellosis is an important consideration in the differential diagnosis of immigrants from Central America presenting with fever or joint complaints. Patients should be specifically asked about ingestion of unpasteurized dairy products. *Thrombocytopenia* (in adults) and elevated transaminases are the most frequent diagnostic clues.

## 249

### ESTIMATING LEPTOSPIROSIS INCIDENCE USING HOSPITAL-BASED SURVEILLANCE AND A POPULATION-BASED HEALTH CARE UTILIZATION SURVEY IN TANZANIA

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The incidence of leptospirosis, a neglected zoonotic disease, is uncertain in Tanzania and in much of sub-Saharan Africa, resulting in scarce data on which to prioritize resources for public health interventions. In this study, we estimate the incidence of leptospirosis in 2 districts in the Kilimanjaro Region of Tanzania using multipliers derived from a population-based health care utilization survey (HCUS) and cases identified through hospital-based sentinel surveillance. We conducted a population-based household HCUS in the Moshi Rural and Moshi Urban Districts in the Kilimanjaro Region from June 13 through July 22, 2011. Wards within the 2 districts were selected randomly with population-weighting; 27 households in each ward were included in the survey. Heads of household were queried about the health care seeking behavior of household members in the event of fever. Febrile illness sentinel surveillance was conducted at 2 hospitals in Moshi from September 17, 2007 through August 31, 2008. Leptospirosis cases were identified among febrile adult and pediatric inpatients using the standard microscopic agglutination test(MAT); confirmed leptospirosis was defined as a ≥4-fold MAT titer rise and probable leptospirosis as any reciprocal titer ≥800. A total of 810 households were enrolled in the HCUS and multipliers were derived based on responses to questions about health care seeking in the event of febrile illness. Of participants enrolled in fever surveillance residing in Moshi Urban and Moshi Rural, 42(7.1%) of 588 met the case definition for confirmed or probable leptospirosis. After applying multipliers to account for hospital selection, MAT sensitivity, and study enrollment, we estimated the overall incidence of leptospirosis to be ~102 cases/100,000 persons annually. In the first study of leptospirosis incidence in Tanzania, we demonstrate a high incidence. Multiplier methods, such as used in this study, may be a feasible method for improving availability of incidence estimates for neglected diseases, such as leptospirosis, in resource constrained settings.

## 250

### IODINE DEFICIENCY IN PREGNANT WOMEN IN RURAL HAITI

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Because of the poor soil and lack of supplemental iodine, we have hypothesized that iodine deficiency in pregnancy may account for the significant developmental delays we identified in children in our rural Haitian community. We collected urine specimens for iodine levels on 26 gravid women in their late second and early third trimester from two rural villages in Haiti, La Croix and La Coup. La Croix is located in the Artibonite Valley and La Coup is a mountain village. Of the 18 women from La Croix, one half of the women had urine iodine levels of less than 150 µg/L. Of the eight women from La Coup, the median level was less than 60 µg/L. In rural Haiti, there is evidence of iodine deficiency among the pregnant women as indicated by urine iodine levels in gravid women in the second and third trimester. The deficiency is more pronounced in the mountain area. Iodine deficiency may be a significant cause of developmental delay in our villages in rural Haiti.

## 251

### IMPACT OF ANTI-RETROVIRAL THERAPY ON HELMINTHS PREVALENCE AND WORM LOAD IN HIV INFECTED PATIENTS IN GABON

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There is anecdotal evidence for both a reduced prevalence of intestinal helminth infection and worm burden, in HIV positive patients receiving anti-retroviral treatment (ART). The aim of this study is to describe possible differences in both prevalence and worm burden of geo-helminthic infections between HIV infected patients who receive ART, versus those who are not. Furthermore, this study aims to identify possible anthelminthic effects of respective anti-retroviral drugs. ART reduces both prevalence and worm burden in HIV infected patients, irrespective of the level of immune restoration. If so, this effect is due to mitochondrial toxicity of drugs leading to damage of helminthic mitochondria. This is a cross-sectional observation study in HIV patients on ART vs ART naïve patients, matched for CD4 counts, attending the HIV clinic (the 'Centre de Traitement Ambulatoire') Lambaréné, Gabon. Furthermore, a prospective observation study is performed in ART naïve individuals who start ART. Patients are analyzed for geo-helminth infections, microfilariasis and schistosomiasis. Worm larvae and adult worms are preserved in formaline for subsequent electron microscopic analysis for mitochondrial toxicity. At this moment, a total of 177 patients are included in the study, 165 in the cross sectional study and 12 in the prospective study. Demographic characteristics for the different patient groups are statistically not different. The prevalence of *Loa loa* infection is significantly lower in the patient group taking ART (11% vs. 33%, p=0.02). Also, there is a trend towards lower prevalence rates of *S. haematobium* (4% vs. 13%, p=0.18) and overall helminth infection (28% vs 60%, p=0.443) in the patients on ART. In conclusion, preliminary results of our study suggest a direct effect of ART on *Loa loa* infection, and possibly on other parasites. Whether this effect is caused by mitochondrial toxicity will be tested at a later stage of this study. Inclusion for this study is still ongoing.

## 252

### LYMPHATIC FILARIASIS RELATED LYMPHEDEMA: A SYSTEMATIC REVIEW OF INTERVENTIONS TO PREVENT OR REDUCE MORBIDITY

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The Global Program for the Elimination of Lymphatic Filariasis (GPELF) was initiated by the WHO in 2000 with its 'first pillar' the goal of interrupting transmission of the disease by 2020. A second pillar aims to prevent or alleviate disability from chronic disease for the approximately 120 million

LF infected persons who remain at risk of developing lifelong morbidity including hydrocele, elephantiasis and lymphedema. Of the 40 million people already affected, about 15 million suffer from lymphedema. As more resources are put towards the second pillar, the interventions used need to be shown to be effective and well evaluated. In this review the effectiveness of interventions for LF related lymphedema are critically appraised and a comparison is made of evidence based prevention and treatment practices in developed countries with the goal of identifying successful practices that are transferrable across economic and cultural borders. Searches of Medline and Scopus databases were conducted in March 2013 using keywords: lymphatic filariasis, elephantiasis, lymphedema, treatment, prevention, management, home care, self care and hygiene. Papers were excluded if they were reviews or reported only on surgical interventions. 309 papers were returned and 25 papers reporting specific outcomes of interventions aimed at reducing limb volume or acute dermatolymphangioadenitis episodes were included. The reference lists of WHO publications, found reviews and other excluded papers were searched for reports of original interventions and a further 9 papers were included. RCT's, interrupted time series studies and case series reports were most commonly used to evaluate drug interventions, education in self care including frequent washing and drying, elevation and exercise, and the use of pneumatic pumps or other health worker applied treatments. Level of evidence of studies was found to be generally poor; however most studies reported improvements when basic lymphedema management was carried out, especially washing and drying of affected limbs. One study indicated that lymphedema education can improve MDA compliance, but this concept requires further investigation. This review will contribute to acceleration of the impact of the second pillar of the GPELF through drawing together knowledge in lymphedema prevention and management approaches in both LF endemic and developed countries.

## 253

### OPHTHALMOLOGICAL FINDINGS FROM A STUDY COMPARING THE EFFECT OF A SINGLE DOSE OF 150 MG/KG IVERMECTIN AND OF 8 MG MOXIDECTIN ON MICROFILARIA LEVELS IN *ONCHOCERCA VOLVULUS* INFECTIONS

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Control of onchocerciasis as a public health problem in Africa relies on annual ivermectin (IVM) mass treatment. Recently, elimination of transmission was added to control programme objectives. Moxidectin (moxi), a veterinary anthelmintic, is in development to assess whether it is safe for mass treatment with an effect on *Onchocerca volvulus* macrofilaria sufficiently higher than IVM's to reduce treatment rounds to elimination and/or to result in elimination where IVM cannot. In the randomized, double-blinded Phase 3 study in areas in Liberia, Ghana and the DRC without IVM mass treatment, a single dose of 8 mg moxi or IVM was given to 978 and 494, respectively, males and females ≥12 years with ≥10 microfilaria/mg skin (mf/mg). Baseline ophthalmological manifestations included symptoms of *O. volvulus* infection (incl. dead microfilaria in the cornea, punctate opacities, cotton wool spots, eye pruritis) as well as glaucoma, cataract, ocular infections, and other problems. More than 10 microfilaria in the anterior chamber (mfAC) were present pre-treatment in 130 (13%) of moxi treated and 75 (15%) of IVM treated. The number of mfAC and live microfilaria in the cornea (mfCL) (mean±SD) in these subjects were, respectively, 26.4±20.0 and 10.2±8.2 among moxi treated and 26.6±18.4 and 9.1±8.5 among IVM treated. MfAC levels (mean±SD) were 20.4±19.4, 4.7±10.9, 0.5±2.4, 0.3±2.2 and 0.1±0.6 at 4 days, 1, 6, 12 and 18 months after moxi treatment and 25.8±22.8, 8.6±17.7, 0.9±5.9, 1.2±6.5 and 1.7±10.1 after IVM treatment, respectively. MfCL levels (mean±SD) were 7.5±6.3, 0.6±2.3, 0.0±0.12, 0.0±0.0 and 0.0±0.2 at 4 days, 1, 6, 12 and 18 months after moxi treatment and 7.0±7.2, 1.2±4.0, 0.2±1.3, 0.0±0.1 and 0.0±0.0 after IVM treatment, respectively. While the skin mf levels and proportion of subjects with detectable skin mf at 1, 6, 12 and 18 months after moxidectin were significantly lower ($p<0.0001$) than after ivermectin treatment, the differences in the change in mfAC between the treatment groups was not significant ($p=0.12$ for month 12).

## 254

### DIFFERENCES IN EOSINOPHIL-RELATED PATHOGENESIS UNDERLIE THE VARIED CLINICAL PRESENTATIONS OF *LOA LOA* INFECTION BETWEEN TEMPORARY RESIDENTS AND THOSE INDIGENOUS TO *LOA*-ENDEMIC AREAS

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Previous studies have suggested that *Loa loa* infections in inhabitants of *Loa*-endemic areas (END) have marked differences in clinical presentation and post-treatment reactions compared to those of temporary residents (TR). Many of these differences are thought to be immune-mediated. To define the underlying pathophysiology of these differences in clinical presentation, we conducted a retrospective analysis of 186 patients with loiasis seen at the National Institutes of Health. Among the 186, 42 were raised in *L. loa*-endemic regions while 144 were visitors to these same regions. The initial clinical presentation differed markedly between the two groups with only 50% of END having a history of Calabar swelling compared to 82 % of TR ($p<.001$). In contrast, the END were much more likely to have had eyeworm (71% compared to 15%, $p<.001$) and were more likely to have microfilaremia (74% compared to 22% in the TR, $p<.001$). The absolute eosinophil counts (AEC) were markedly different between the groups; the geometric mean (GM) AEC in TR were 1532/uL compared to 706/uL in the END ($p=.0003$). Along with the quantitative increase in AEC, individuals in the TR group showed evidence of increased eosinophil activation in that the serum levels of all four eosinophil granule proteins (EDN, EPO, ECP, and MBP) at baseline were higher in the TR group compared to END. In addition, the levels of EDN and EPO in the TR group at presentation were positively correlated with both the AEC and IgE levels ($p <.05$). Baseline serum levels of eosinophil-associated cytokines, including IL-4, Eotaxin, GM-CSF, and IL-5 were all found to be elevated in the TR group compared to the END group ($p<0.05$ for all cytokines). These data extend earlier observations related to immunologically based differences between TR and END of Loa-endemic regions. Additional data concerning eosinophil-related pathogenesis of loiasis and changes in AEC and eosinophil-related cytokines following treatment will be discussed.

## 255

### *LOA LOA* INFECTION MODULATES THE T CELL MEMORY RESPONSE TO MYCOBACTERIA

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Chronic filarial infections have been associated with a type of bystander suppression felt to be mediated by IL-10-producing Th2 or adaptive

T regulatory cells. To assess the heretofore unexplored dynamics of homeostatic and antigen (Ag)-specific memory T cell compartments in the context of filarial infection, including the recently described memory T stem cell compartment --reported to persist stably long-term -- we used multiparameter flow cytometry on PBMCs from 17 microfilaremic *Loa loa* (Ll) -infected (Loa+) and 10 Ll–uninfected (Loa-) subjects following stimulation with filarial (BmA) or mycobacterial (PPD, ESAT-6, CFP10) Ag. Using intracellular CD154 to mark Ag-activated CD4+ T cells, we demonstrated that the Loa+ group compared to the Loa- group showed an increase in CD3+ CD4+CD154+IL-10+ cells (Median increase on stimulation (M)=0.1230% vs -0.14%, p=0.03) in response to BmA as well as to PPD (M=0.1237% vs. -0.2583%, p=0.009), and an increase in CD4+CD154+IL-4+cells with CFP10 (M=0.3% vs. 0%, p=0.0266). Loa+ subjects showed no expansion of CD4+CD154+CD45RO+IL-4+, IL-10+ or IL4+IL-10+ producing central memory (Tcm, CCR7+CD27+), effector memory (Tem CCR7-CD27-), transitional memory (Ttm CCR7-CD27+) or stem cell memory (Tscm, CD45RA+CCR7+CD27+CD95+) cells at homeostasis or on stimulation with either BmA or mycobacterial Ag. However, an increased frequency of a poorly studied antigen-experienced population of CD4+CD154+CD45RA+CCR7+CD27+CD95-IL-4+ producing cells were seen in Loa+ individuals in response to BmA (M=0.15% vs. 0.056 %, p=0.01) as well as to PPD (M=0.1% vs. 02%, p=0.03) and CFP10 (M=0.15% vs. 0.14%, p=0.007) compared to the Loa- subjects. These data suggest that CD154 can be used to quantify Ag activated CD4+Th2 responses to parasitic and bystander Ag. We also have been able to identify a novel Ag-experienced CD45RA+ cell population capable of producing Th2-related cytokines that may be a new CD4+ 'memory' cell capable of modulating the effector T cell response to parasite and bystander Antigens.

## 256

### *IN VITRO* VALIDATION STUDY OF PEPTIDES FROM IN SILICO ADVANCED EPITOPE MAPPING PREDICTIONS TO DELINEATE T EFFECTOR EPITOPES WITHIN STAGE-SPECIFIC *BRUGIA MALAYI* SECRETED PROTEINS

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The complex host-pathogen interactions between humans and the causative agents of lymphatic filariasis--*Wuchereria bancrofti, Brugia malayi*, and *Brugia timori*--are well known to favor the survival of both host and pathogen. However, the quality of life for chronically infected humans can be severely affected by the disabling disfigurements manifesting upon death of adult filarids. Efforts continue to be made for the development of an effective vaccine to combat these pathogens. Nonetheless, it is the same host-pathogen relationship that makes the traditional approach to vaccine design, with respect to immunogenic antigen discovery, extremely difficult to apply against the multicellular filarids. Thus, an alternative approach to the discovery of immunogens was the foundation of this study. This approach consisted of screening pathogen proteins with immuno-informatics algorithms and testing predictions with immunological assays. Over 900 protein sequences believed to be secreted during the L3, adult male, adult female and/or microfilarial life stages of *B. malayi* were screened. A peptide library of 20 putatively immunogenic epitope clusters was created and tested for proof-of-concept using a 10 day re-stimulation assay on naive peripheral blood mononuclear cells (PBMCs) with different peptide concentrations ranging from 25mg/ml to 200mg/ml. In response to peptide stimuli, cytokine specific secretions were assessed by ELISpot assays. Specific responses, such as interleukin-4 and interleukin-5 secretions, varied among the different patient PBMCs samples. Optimal cytokine production also varied among different peptide concentrations where a subset of peptides yielded the strongest cytokine secretion at lower concentrations. Results demonstrate the potential of immuno-informatics software as an alternative, low cost approach to discovering filarid immunogens as well as the feasibility of testing predictions with immunological assays to further characterize the specific effector T cell responses by cytokine markers indicative of CD4+ T cell recognition.

## 257

### H1 RECEPTOR BLOCKADE ENHANCES EOSINOPHIL-MEDIATED CLEARANCE OF FILARIAL NEMATODES

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Recently, we demonstrated that chronic administration of fexofenadine, an H1-receptor blocker, reduces adult worm burdens in BALB/c mice infected with Litomosoides sigmodontis, a tissue-invasive filarial infection of rodents that lives for months in immunocompetent BALB/c mice. In this study, we sought to determine the mechanism by which this occurs. In vitro addition of histamine or fexofenadine to L3 stage worms did not alter worm motility or duration of survival in culture, suggesting that fexofenadine does not directly affect the viability of L3 worms. To evaluate the *in vivo* effects of fexofenadine, we infected mice by subcutaneous injection of 40 L3 stage worms and treated BALB/c mice with an average of 20 mg/kg per day of fexofenadine administered in drinking water. Consistent with our previous studies, worm counts dropped significantly in fexofenadine treated animals (mean number adult worms = 18 in untreated mice vs 5 in fexofenadine treated animals, p = 0.001). Next, we conducted multicolor flow cytometry to quantify eosinophil numbers in the pleural space, the site of adult worms. In contrast to untreated mice, in which mean eosinophil count in the pleural space was 0.6 x 106, fexofenadine-treated mice had 2.4 x 106 pleural space eosinophils. To determine if eosinophils were important for fexofenadine-treated mice, we next evaluated whether fexofenadine decreases worm burden in eosinophil-deficient dblGATA mice. Eosinophil deficient mice exhibited similar numbers of adult worms 8 weeks post-infection as wild-type mice (mean 14 adult worms per mouse in both groups). In contrast to studies in wild type mice, fexofenadine treatment did not alter adult worm burden in dblGATA mice (mean 17 adult worms per mouse, p compared to untreated dblGATA mice = 0.4). These results suggest that fexofenadine enhances clearance of adult filarial worms by augmentation of the eosinophil response.

## 258

### EVIDENCE FOR INVOLVEMENT OF CYS-LOOP LIGAND-GATED ION CHANNEL GENES IN REPRODUCTION OF FILARIAL WORMS

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Several commonly used anthelmintic drugs (macrocyclic lactones or MLs, and nicotinic agonists) target members of the CLGIC gene family. MLs (e.g., ivermectin (IVM) which binds to glutamate chloride-gated channels, GluCls) and nicotinic agonists (e.g., levamisole which binds to nicotinic acetycholine receptors, nAChR), have their greatest activity against microfilariae (Mf) of filarial nematodes such as *Onchocerca volvulus*. These drugs also affect fecundity in some species. This suggests that GluCl and nAChR may have a role in reproduction, but the nature of that role is poorly understood. Our prior studies have shown that gene expression patterns in filarial nematodes often correlate with biological function. We used *in situ* hybridization to study gene expression patterns for seven CLGIC genes including IVM-sensitive subunits from GluCls and levamisole-sensitive receptor from nAChR in the filarial parasite *B. malayi*. Six of these genes were strongly expressed in early developing embryos (morula and pretzel stage) in the uterus and spermatogonia in testis, weakly expressed in later stage embryos, and partially or not expressed in stretched microfilariae or mature sperm. Most of these genes were strongly expressed in the wall of the uterus and vas deferens adjacent to stretched Mf or mature spermatozoa. Some of these genes had unique expression patterns. For example, AVR-14B, which encodes an IVM-sensitive GluCl channel subunit, was only expressed in female worms, and Bm1_40515,

a member of cAChR gene family was only expressed in male worms; Bm1_35890, a *C. elegans* orthologue of *unc-29* encoding the levamisole-sensitive receptor was strongly expressed in both male and female worms. In summary, *in situ* localization results are consistent with the observed suppressive effects of IVM and levamisole on embryogenesis in *B. malayi* and provide the molecular evidence for involvement of CLGLC genes in filarial reproduction. Our studies have also provided new insights regarding the sites of action of MLs that are broadly active against nematodes and arthropods.

## 259

### WHOLE GENOME SEQUENCING OF *WUCHERERIA BANCROFTI* FROM AN INFECTED HUMAN BLOOD SAMPLE

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Lymphatic filariasis (LF) is a major threat to human health in the tropics, leading to the disfiguring and debilitating conditions hydroceles and elephantiasis. There are approximately 1.2 billion people living in LF endemic countries where 120 million people currently suffer from the disease. The parasites that cause LF are separated into two genera, *Brugia* and *Wuchereria*, with *W. bancrofti* (Wb) responsible for ~90% of LF cases. *Wb* has been neglected in genomic studies due to complications in sample collection and preparation, such as lack of adult stage samples. Therefore, in order to obtain sufficient material to perform genome sequencing Wb larval stages (microfilaria) must be sampled from infected host blood. These samples will inevitably contain human leukocytes and therefore human DNA contamination. Here we present a method to reduce human DNA contamination and subsequently produce high quality genome sequence for Wb directly from an infected human blood sample. Twenty-five patients were selected to have varying concentrations of mircofilaria in the blood. For each patient we used a set number (1, 2, or 3) of Percoll density gradient centrifugation steps to remove cells containing human DNA. After DNA extraction we quantified the concentration of human to Wb DNA by way of qPCR. We determined that only a single Percoll treatment was needed to optimize reduction of human DNA while still maximizing the recovery of Wb DNA. Successive treatments decreased the yield of Wb DNA while having minimum effect on human DNA concentration. We selected a single treated sample for whole genome sequencing based on the results of qPCR. Whole genome sequenced was performed on the Illumina HiSeq2000 platform that generated 200-million paired-end reads of 150 base pairs each. The resulting sequences were mapped to human reference Hg19 and the previously assembled contigs of Wb from Mali. Human DNA comprised only 40% of our sequence reads with remaining reads belonging to a combination of Wb (58%) and its bacterial symbiont, *Wolbachia* (2%). Removal of human DNA resulted in an average nucleotide coverage of 200x across the Wb genome, which is 30-50 times higher than that currently available for the Wb genome from Mali. This sequence of the Wb genome is unique in being derived from an individual infected whole blood sample and is the first Wb genome available from Papua New Guinea.

## 260

### DISCOVERING NOVEL PARASITICIDES FROM FILAMENTOUS FUNGI

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Mycosynthetix owns one of the largest libraries (55,000 strains) of filamentous fungi in the world. These organisms were isolated from substrates sourced from a wide variety of ecosystems and include many strains from tropical rain forests where there is a high degree of biodiversity. Our primary purpose is to exploit the medicinal and pharmaceutical potential of this large library. The targets of the screening efforts include cancer, bacteria, fungi and several human and animal parasites. The latter include malaria, trypanosomes, leishmania, soil transmitted helminths (STHs) and filarial worms which infect humans and animals. We have established validated whole organism assays to determine activity of both pure compounds and semi-purified extracts of fungi against GI tract nematodes, filarial worms, adult arthropods and mosquito larvae. Pure compounds have been shown to have high activity against one or more of the following; *Haemonchus contortus*, *Cooperia onchophora*, *Strongyloides stercoralis, Brugia pahangi* (Bp) and *Dirofilaria immitis* (Di) microfilaria (Mf) and L3 and L4 stages and *Aedes aegypti* larvae. There is little crossover of activity between the endo- and ectoparasites. Several compounds not only had high potency against BpMf but also caused high mortality within 24 hours. A small library of carefully selected fungal metabolites was tested in the BpMf assay at 50 ppm giving an overall hit rate ($ED_{100}$) of 96.1% at 120 hrs. Upon down titration, 30/58 compounds were fully active at 3.125 ppm (3-6 μM) or less and a number had nanomolar activity. Several of these 30 were also active against DiMf. One compound showed potent activity against Bp L3 and L4 and DiL3, and several others are being tested. Results from several mammalian cell lines coupled with associated anti-parasitic data have identified a number of compounds with high potency and a promising *in vitro* therapeutic index.

## 261

### COEVOLUTION AND PARTNER FIDELITY IN PARASITE - BACTERIAL SYMBIOSES

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Parasite - bacterial interactions are common in nature, and in particular, beneficial symbiotic interactions between these organisms can have large impacts on parasitic diseases and parasite biology, ecology and evolution. Therefore it is essential that we understand the fundamental pressures that influence the formation and maintenance of parasite - bacterial symbioses to fully comprehend how these interactions contribute to the evolution of clinically relevant parasites (e.g. filarial nematodes). Toward this, we utilized the *Steinernema* nematode - *Xenorhabdus* bacteria model system to study the influence of bacteria on parasitic success and the evolution of parasite -bacterial associations. We found co-cladegenesis (congruence) between the phylogenies of *S. feltiae* nematode isolates and their natural *X. bovienii* bacterial symbionts, which are considered strains of the same species by current metrics. Robust co-cladegenesis indicates that there is strong selection for maintenance of the symbiosis and that coevolution is likely occurring. To test this possibility we experimentally measured the impact of the bacterial strains on the relative success of parasitic infection and were able to detect differences between the nematode - bacterial combinations, indicating that bacterial strains may differ in their contributions to parasitic infection success. In addition, the differences reflected a pattern that is consistent with coadaptation occurring at a sub-species level. The better success of parasites associated with native partners, relative to non-native partners, could serve to reinforce partner fidelity between the pair, and this in turn can influence the larger phylogenetic patterns that we observe. This suggests that interactions between bacterial strains and parasites may impact the parasite evolution. In addition, our data highlight that interactions between parasite and bacterial populations likely differ and should be considered in our studies of the impacts of bacteria on parasite biology and parasitic diseases.

## 262

### RAPID SCALE UP WITH HIGH TREATMENT COVERAGE FOR LYMPHATIC FILARIASIS ELIMINATION IN MALAWI: IMPACT ON INFECTION RATES AFTER 3 TO 4 TREATMENT ROUNDS

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Lymphatic filariasis (LF) caused by *Wuchereria bancrofti* is highly endemic and widespread in Malawi where 26 of the 28 districts are endemic. The national LF elimination programme was launched in 2008 to protect 14 million people at risk of acquiring the infection. During 2008, 2.7 million people were treated in eight of the 26 endemic districts with a reported drug coverage of 80.5%. In the following year, MDA scaled up dramatically to achieve 100% geographic coverage and 10.8 million people were treated. Treatment coverage was 80% or higher for the subsequent treatment rounds carried out in all endemic districts. To test the hypothesis that rapid scale-up and high treatment coverage accelerates the transmission interruption process we conducted sentinel site surveys to monitor impact on infection rates. Mapping surveys to identify implementation units for MDA were conducted in 2000 and 2003 with ICT cards and the results informed the identification of 15 high risk villages which serve as sentinel sites for impact assessment. The overall microfilaremia (MF) baseline rates from 4738 people tested was 1.6%, ranging from 0.5% in Zomba in the Southern Region, to 9.1% in Karonga in the Northern Region. The Overall MF rate decreased by 87.5% after 3-4 rounds of MDA and MF was not detectable in 10 of the 15 sentinel sites where 5263 people were tested in 2012. Only one of the sentinel sites had MF rate above 1%. All endemic districts in Malawi will conduct their 5$^{th}$ or 6$^{th}$ annual MDA in 2013. If the high treatment coverage is maintained and MF prevalence continues to decrease, all sentinel sites will qualify for transmission assessment surveys to determine whether to stop mass treatment.

## 263

### CRITERIA FOR TESTING DEVELOPMENT OF RAPID DIAGNOSTIC TEST TO SUPPORT ONCHOCERCIASIS CONTROL AND ELIMINATION PROGRAMS

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There is often significant discrepancy between analytical performance of diagnostic tests determined under laboratory conditions and operational performance of the same tests. Failures induced by real-world circumstances may compromise test performance and subsequent availability to the communities in need. In order to address potential failures from the research and development phase through final product release, we have developed data-supported criteria that are relevant to successful test deployment into a field setting. We have applied these criteria to the development of a rapid test for exposure to the parasitic worms *Onchocerca volvulus* (Ov) which causes onchocerciasis, or river blindness. A major cause of preventable blindness around the world, Ov is transmitted to humans through the bite of the blackfly and typically affects poor, rural communities near fast-flowing streams and rivers. Mass administration campaigns of the drug ivermectin have significantly reduced disease burden in some regions to the extent that elimination has become possible. To enhance surveillance of onchocerciasis exposure and infection, we have developed rapid diagnostic test (RDT) prototypes of a clinically-validated assay detecting IgG4 antibodies specific to a previously validated Ov antigen, Ov16. These RDTs have been advanced to readiness for field studies through evaluation with a collection of development criteria designed to anticipate common user failures, operational testing challenges, and options in sample collection. We describe the development of a positive control human anti-Ov16 monoclonal IgG4 for use by the onchocerciasis community and our performance and stability data for application to quality control and assurance of Ov16 RDTs. We present data informing the development criteria, usability feedback, development of instructional material, and performance of the test through induced failure modes and rigorous storage and operational conditions. All of these measures ensure that a robust and user-friendly Ov16 test is developed and brought to the field.

## 264

### IMPACT OF REPEATED IVERMECTIN TREATMENTS AGAINST ONCHOCERCIASIS ON THE TRANSMISSION OF LOIASIS: AN ENTOMOLOGICAL EVALUATION IN CENTRAL CAMEROON

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Mass treatments with ivermectin (IVM) against onchocerciasis started in Cameroon in the early 1990s. In the Lekie division, distributions with low coverages started in 1993, but it was not until 1999 that the whole area was covered using the community-directed treatment with IVM (CDTI) strategy. In this area, onchocerciasis is coendemic with loiasis (*Loa loa* filariasis) and, since 1995, cases of serious adverse events (SAEs) were recorded in patients presenting a high *Loa* microfilaremia (>30,000 microfilariae per mL blood). An entomologic study on loiasis was conducted from May 1999 to April 2000 in one village of the area (Kokodo). Chrysops were caught during 3 consecutive days every week to assess the proportions of flies harboring *Loa* larvae of any stage (infection rate), stage 3 larvae (L3) in the head (infective rate, IR), and L3 whatever the location (potential infective rates, PIR). The mean numbers of L3 per infective and per potentially infective fly (MHL3 and MFL3) were also assessed. A second entomologic study was carried from March to June 2012 to evaluate the impact of 13 years of CDTI on the transmission of loiasis. The sites and methods were identical to those used in 1999-2000. The indices measured during this 4-month period were compared with those obtained during the same months in 1999-2000. The biting density was almost three-fold higher in 2012 than in 1999-2000. There was a significant reduction in the infection rates between the two periods (from 7.1 to 3.0%, p=0.001). The differences in the IRs, PIRs, MHL3 and MFL3 were not significantly different between the two periods. The infection rate remains high in spite of its significant reduction, and the stability observed in the other indices after 13 years of CDTI shows that transmission of L. loa is still active in the area. A parasitologic survey should be conducted to evaluate whether these entomologic results correspond to high levels of infection in the population.

## 265

### DISCOVERY PROTEOMICS: UNRAVELLING THE MUTUALISTIC SYMBIOSIS OF *WOLBACHIA* AND THE FILARIAL NEMATODE *BRUGIA MALAYI*

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The parasitic nematode *Brugia malayi* is a causative agent of lymphatic filariasis, a disfiguring disease affecting over 120 million people worldwide. *B. malayi* exists in a mutualistic symbiotic relationship with the α-proteobacterium *Wolbachia*. We have applied a global proteomic profiling approach to investigate the molecular basis of this symbiosis. Adult female *B. malayi* in the mammalian host *Meriones unguiculatus* were sampled at multiple time-points post-antibiotic depletion. Deep proteome mining combined with high-resolution mass spectrometry was used for comprehensive proteome profiling of *Wolbachia*/worm at these selected time-points. *Wolbachia*/worm ratios were also monitored by qPCR. In-solution tryptic proteolysis coupled with reversed phase liquid chromatography and analysis by high-resolution mass spectrometry provides a powerful tool for global proteome profiling. This initial shot-gun approach has been optimised to include an extensive peptide pre-fractionation step using the Agilent 3100 OFFGEL fractionator system. Using a combination of pre-fractionation and established proteomic workflows we observed improved proteome coverage by an increase in peptide/protein identification. Following basic analysis through established bioinformatics pipelines, transcriptomic, proteomic, and published datasets will be integrated in a systems biology approach with the objective of understanding the molecular basis of the mutualistic *Wolbachia*/*B. malayi* symbiosis.

## 266

### A•WOL MACROFILARICIDAL DRUG DISCOVERY - LEAD OPTIMIZATION OF HIT CHEMOTYPES WITH ANTI-*WOLBACHIA* EFFICACY IDENTIFIED DURING THE SCREENING CAMPAIGN OF FOCUSED ANTI-INFECTIVE AND DIVERSITY-BASED LIBRARIES

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There is an urgent need to develop a novel treatment for filariasis, and targeting *Wolbachia* provides safe macrofilaricidal activity with superior therapeutic outcomes compared to standard anti-filarial treatments. In order to turn this into a public health tool suitable for control programs, the A•WOL Consortium utilises *in vitro* cell and nematode assays, followed by *in vivo* assays, to screen chemical libraries for anti-*Wolbachia* activity. During the screening campaign, the *Wolbachia in vitro* assay has been developed using Operetta high content imaging with automation, in order to radically increase throughput. Using this approach, screening of 10130 compounds, from libraries of focused anti-infectives and registered drugs, has identified 632 hits, with 324 showing improved efficacy over doxycycline. The diversity-based approach has involved procurement and ongoing screening of >510k compounds from large libraries. Progression along the pipeline selects for hits which also show activity against nematode *Wolbachia in vitro* and *in vivo*. Hits are then scrutinised to determine suitability for structure-activity relationship assessment, and narrow-spectrum anti-*Wolbachia* activity in order to select the best candidates to take forward. These extensive screening activities have generated four independent lead series chemotypes based on equivalent or improved activity over doxycycline in *in vitro* and *in vivo* models (as absolute potency or duration of treatment to deliver *Wolbachia* elimination), chemical tractability and prior experience with the class. These lead series are progressing through a rigorous lead optimisation and candidate selection process, using iterative cycles of medicinal chemistry and biological testing in order to deliver at least one novel pre-clinical candidate and a chemically distinct back-up, aligned with our Target Product Profiles for an anti-*Wolbachia* macrofilaricide. Ongoing screening activities aim to provide additional, chemically diverse hits, with one-order improvement in absolute potency or significant shortening of treatment (≥25%).

## 267

### DEVELOPMENT OF A FILARIAL MURINE INFECTION MODEL TO SCREEN ANTI-*WOLBACHIA* DRUGS

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Filariasis is a priority neglected tropical disease affecting >150 million of the World's poorest populations. New drugs require testing *in vivo* if control of filariasis is to be sustained. It is possible to passage filariae of the genus Brugia in gerbils including the human pathogen, *Brugia malayi*. However inbred lab mice are refractory to full development of B. malayi. It would be desirable to establish a robust murine model of Brugian filariasis to screen new pharmacological agents, including anti-microbials that target the nematode endosymbiont, *Wolbachia*. The advantages of this include: reduced costs of manufacturing sufficient drug for testing, lower animal husbandry costs and use of a rodent with a well-characterised pharmacokinetic profile. Previously it has been reported that Brugia spp consistently develop through to fecund adult infections in the peritoneum of lymphopenic mice, such as Severe Combined Immuno-Deficiency (SCID) mice. Our own experimentation has identified that modulating eosinophil function greatly enhances survival of larvae. Therefore we tested male mice with impaired eosinophil responses (interleukin-4 receptor alpha; IL-4Rα-/-, IL-4Rα-/-IL-5-/-) and SCID mice, all on a BALB/c background, for their permissiveness to fecund Brugia infection. Whilst all strains were permissive at +5 weeks (juvenile adult stage) typically yielding between 20-40% recoveries of initial inoculate, adult infections of B. malayi waned past this stage in IL-4Rα-/- , so much so that IL-4Rα-/- inconsistently retained permissiveness to fecund infections. Contrastingly both IL-4Rα-/-IL-5-/- and SCID maintained 10%-20% recoveries of microfilariae (mf) producing Brugia adults to beyond +20 weeks. Therefore IL-4Rα-/-IL-5-/- and SCID were validated for anti-*Wolbachia* drug screening using oral administration of tetracyclines. *Wolbachia* expansion during L3 to L4 development could be successfully blocked in both strains by oral daily tetracycline administration. Also both strains could be successfully used to deplete *Wolbachia* by >1log (a level of permanent sterilisation) and prevent mf release in adult female Brugia after 4-6 weeks oral administration of tetracycline. Thus we have demonstrated that selective deficiency of adaptive immune responses controlling eosinophil recruitment or SCID can yield a highly permissive mouse model of filariasis validated for anti-*Wolbachia* pre-clinical screening.

## 268

### INTESTINAL HELMINTHIASIS DIAGNOSED IN DAKAR, SENEGAL

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Parasitic infections, caused by intestinal helminths and protozoan parasites, are among the most prevalent infections in humans in developing countries. In developed countries, protozoan parasites more commonly cause gastrointestinal infections compared to helminths. Intestinal parasites cause a significant morbidity and mortality in endemic countries. Helminths are worms with many cells. Nematodes (roundworms), cestodes (tapeworms), and trematodes (flatworms) are among the most common helminths that inhabit the human gut. The goal of this study was to

determine the prevalence of digestive helminthiasis among patients referred to the laboratory of parasitology and mycology at Le Dantec hospital in Dakar for examination of stool samples from 2004 to 2009. Out of 1 526 direct stool examinations (Ritchie and Baerman techniques) analyzed at the laboratory of parasitology and mycology of Le Dantec hospital from 2004 to 2009, 310 were positive for intestinal helminthiasis (20.3%). The main species found were: *Ascaris lombricoïdes, Trichuris trichiura, Strongyloides stercoralis, Taenia saginata* and *T. solium*. Most of the patients had a single parasite (90.1%, versus 9% with two and 0.9% with three). Men are infected more often than women, accounting respectively for 58% and 42% of the infections, for a sex ratio of 1.38. Children aged 10 to 15 years had the highest prevalence of infection: 34.5%. The results show that digestive helminthiasis is endemic in Dakar, where it is necessary to implement: deworming campaigns, health education and environmental improvement.

## 269

### PREVALENCE OF SOIL TRANSMITTED HELMINTHS AFTER MASS ALBENDAZOLE ADMINISTRATION IN AN INDIGENOUS COMMUNITY OF THE MANU JUNGLE IN PERU

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Few data are available on soil transmitted helminths in indigenous populations in the Amazon. Albendazole mass administration has not been carefully evaluated in that context. We report the prevalence of soil transmitted helminths, anemia, and malnutrition in a Matsigenka ethnic group from Yomibato in the Peruvian Amazon rain forest. All participants received 2 doses of albendazole on consecutive days, 3 months and again 2 weeks, before data collection. In total, 290 subjects from 52 families were studied. Most were female (53%), 22% were under 5 years old, 39% between 5 and 19 years, 22% between 20 and 35 years, and 17% were older than 35 years. *Trichiuris* (30%), hookworm (19%), *Ascaris* (18%), and *Strongyloides* (6%) were the most common helminths. *Enterobius* (4%), *Fasciola* like eggs (2%), *Capillaria hepatica* (2%), and *Hymenolepsis nana* (1%) were also found. Mean (±SD) egg counts/gram of stools were 1,194 (±1,821) for *Ascaris*, 100 (± 132) for *Trichiuris*, 31 (± 17) for hookworm. *Giardia* (28%) and *B. hominis* (46%) were common. Most (63%) participants had at least one parasite other than *B. hominis* and 50% had helminths. Subjects 5 to 19 years (53%) and 20 to 35 years (67%) old had helminths more often than those under 5 years (36%) and older than 35 years (41%) ($p=0.02$). One third (35%) of participants had anemia, which was more common in children under 5 years than in other participants (67% vs. 25%, $p< 0.001$). Stunting in children 0 to 20 years was common (70%), but wasting was not (3%). Children 0 to 10 years old were often underweight (29%). Overall, 72% of children were malnourished. *Ascaris* and *Trichiuris* mean egg counts were higher in malnourished children than in the rest, but differences were not significant ($p>0.05$). Despite repeated doses of albendazole, a higher than expected prevalence of soil transmitted helminths was found. Although, infection intensity was low, malnourished children tended to have higher egg counts. Anemia was common and associated with age.

## 270

### MODULATION OF MYCOBACTERIAL ANTIGEN-SPECIFIC MONO- AND MULTI-FUNCTIONAL TH1, TH2 AND TH17 CELLS IN LATENT TUBERCULOSIS BY CO-EXISTENT HOOKWORM INFECTION

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It is estimated that tuberculosis (TB) infects one third of the world's population, resulting in two million deaths per year. In addition, helminth infections are estimated to occur in 1.5 billion people worldwide and a great majority of those infections are concentrated in developing nations where TB is endemic. A hallmark of helminth infections, both in experimental models and human infection, is the generation of profound T helper (Th) 2 and T regulatory cell responses that have the potential to suppress the predominant Th1 (IFN-γ-mediated) response needed to control *Mycobacterium tuberculosis* (Mtb) infection and enhance susceptibility to infection and/or disease. Given the overlapping distribution of hookworm infections and tuberculosis in a country like India, we wanted to determine the role of coincident hookworm infection on responses at steady state and on Mtb - specific immune responses in latent tuberculosis (LTB). We examined the cellular responses in individuals with latent TB with or without concomitant hookworm infection. By analyzing the expression of Th1, Th2 and Th17 subsets of $CD4^+$ T cells, we were able to demonstrate that the presence of coincident hookworm infection significantly diminished both spontaneously expressed and Mtb - specific mono - and dual - functional Th1 (IL-2, TNF-α and IFN-γ) and Th17 cells (IL-17A, IL-22 and IL-10). Hookworm infection, in contrast, was associated with expanded frequencies of mono - and dual - functional Th2 cells (IL-5, IL-4 and IL-13) at both steady state and upon antigen - stimulation. This differential induction of $CD4^+$ T cell subsets was abrogated upon mitogen stimulation. In addition, coincident hookworm infection was associated with increased adaptive T regulatory (aTreg) cells but not natural regulatory T cells (nTregs) in latent TB. Finally, the $CD4^+$ T cell cytokine expression pattern was also associated with alterations in the systemic levels of Th1 (IFN-gamma and TNF- α) and Th2 (IL-5 and IL-13) cytokines. Thus, coincident hookworm infection exerts a profound inhibitory effect on protective Th1 and Th17 responses in latent tuberculosis and may predispose toward the development of active tuberculosis in humans.

## 271

### DETECTION OF *STRONGYLOIDES STERCOLARIS* IN FECAL SAMPLES USING CONVENTIONAL PARASITOLOGICAL TECHNIQUES AND REAL TIME PCR: A COMPARATIVE STUDY

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Strongyloidiasis is an extremely common cause of morbidity and mortality worldwide. In Egypt, the prevalence rate ranges from 1.5-11% with the increasing number of immunodeficient individuals, investigating the epidemiology and outcome of *Strongyloides stercoralis* infection is essential. Conventional diagnostic techniques don't efficiently detect the parasite. Therefore, the need for more efficient methods that improve diagnosis particularly in those at risk to develop the severe form of the disease is warranted. Stool samples were collected from 115 patients living in rural areas in Ismailia governorate, Egypt. All samples were subjected to agar plate culture (APC), Harada-Mori culture, Baermann concentration, formalin ethyl acetate concentration (FEAC), and real-time PCR targeting the small subunit of the rRNA gene. Among the stool samples analyzed, *S. stercoralis*: Harada-Mori detected 11 positive samples (9.6%), FEAC detected 13 (11.3%), Baermann concentration detected 16 (13.9%) and APC detected 18 (15.7%) samples. Real-time PCR assay detected *S. stercoralis* DNA in 23 (20%) samples. One sample was positive by APC but negative by the other parasitological methods. This sample was confirmed positive by real time PCR. Real-time PCR is a very sensitive and specific method, offering nearly a two-fold increase in the detection rate of *S. stercoralis* by FEAC. It doesn't require much time to perform (45 min for extraction and about one hour in amplification and detection), reduces the risk of infection, has the ability to detect dead larvae and easy to perform and interpret the data, but it still the most expensive method. On the other hand, real-time PCR technology is becoming available in an increasing number of centrally located research centers within low to middle-income countries. Moreover, PCR has the ability of detecting multiple pathogens simultaneously in one test using multiplex real-time PCR, thus saving time and money.

## 272

### *TRICHINELLA* SERINE PROTEASE INHIBITOR INHIBITING NEUTROPHIL ELASTASE MIGHT DIMINISH NEUTROPHIL-MEDIATED INFLAMMATION

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*Trichinella spiralis*, a tissue nematode, is an important zoonotic helminth causing trichinosis in human. To survive in their host, the parasite larvae penetrate host skeleton muscles and induce host microenvironment for cyst formation. Additionally, the larvae secrete several biological molecules that exert their roles in host-parasite interaction by modulation and inhibition of host inflammation and immune responses. The serine protease inhibitors commonly found in the larval stage of *T. spiralis* (TsSerp) are suspected secretory proteins. In previous studies, serpins from other pathogens mainly inhibit neutrophil serine proteases [NSPs: Elastase (NE), Proteinase 3 (PR3), and Cathepsin G (CG)] that are crucial mediators in inflammatory responses. However no available data have been suggested for the role of TsSerp in neutrophil-mediated inflammation. In this study, we aimed to elucidate roles of TsSerp in NSPs inhibition and neutrophil-mediated inflammation. Recombinant TsSerp (rTsSerp) was heterologously expressed in M15 strain *E. coli* and purified using $Co^{2+}$ affinity column. The covalent complexes between TsSerp and NSPs were analyzed using reducing SDS-PAGE and western blot. To determine inhibitory activity of TsSerp, specific fluorogenic substrates for NSPs were applied into NSPs/TsSerp reactions and measured an activity with the fluorometer. Our results demonstrated that rTsSerp was successfully expressed in bacteria at molecular weight of 44 kDa. The covalent complex strongly appeared in combination between rTsSerp and NE with dose- and time-dependence but weak when mixing with PR3 and CG. Additionally, rTsSerp could initially inhibit NE to cleave a specific fluorogenic substrate at the enzyme:inhibitor (E:I) ratio of 1:1 and completely inhibit at 1:4. Unfavorably, rTsSerp was not a good inhibitor for PR3 and CG because it could not inhibit their activity even E:I ratio reached to 1:10. These findings suggested that rTsSerp was only specific to NE. Currently, we are determining the inhibitory role of TsSerp in neutrophil-mediated inflammation. We strongly hope that understanding inhibitory roles of TsSerp will benefit drug and vaccine development against trichinosis and might therefore be a valuable therapeutic modality to treat a large spectrum of chronic inflammatory, autoimmune, and allergic diseases in the future.

## 273

### TRICHINOSIS IN ARGENTINA: A 40-YEAR STUDY OF SYMPTOMATOLOGY AND THERAPEUTIC RESPONSE

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Trichinosis is a disease caused by nematodes of the genus trichinella. It is acquired by consumption of undercooked pork meat. *Trichinella* is endemic in certain areas of Argentina, and *T. spiralis* is the most common species. It presents in outbreak fashion, and these can happen any time of the year. In the following study we aimed to characterize the symptoms during different outbreaks of trichinosis and the therapeutic response to different treatments. Retrospective study of 1020 patients with clearly positive epidemiological correlation for trichinosis from the last 40 years at hospital Rawson in Cordoba, Argentina. Patients underwent epidemiologic assessment, physical examination, and complete blood count with differential (CBC). In the last 22 years patients had measurement of creatine-phosphokinase (CPK). Direct immunofluoresce was used for diagnosis of the "Sentinel" case (first presentation of an outbreak), and the rest of the cases associated to each outbreak were based on epidemiological association. Response to therapy was evaluated by improvement of symptoms, CBC and CPK (when applicable). Of the 1020 patients, 89% had typical "toxic symptoms" of trichinosis (headache, weakness, fever), 80% had ocular pain, 78% myalgia, 40% edema of lower extremities, and 43% gastrointestinal symptoms (diarrhea or constipation). Three patients had skin rash and one patient developed meningoencephalitis. 210 patients were treated with thiabendazole. Of these, 64% developed nausea and vomiting as adverse effects (which subsidized upon stopping the drug). Normalization of CBC and symptoms occurred at 4 months in 75% of patients in this group. 90 patients were treated with mebendazole, none of them had adverse effects from the drug. Normalization of CBC and symptoms was achieved at 6 months in 71% of patients in this group. 720 patients were treated with albendazole. There were no significant adverse effects in this group and 90% of patients showed symptomatic recovery at 48hours with normalization of CBC at 45 days. In conclusion, our 4-decade experience with trichinosis in Argentina, we found that the most commons symptoms were the so-called "toxic symptoms", myalgia and ocular pain. These symptoms vary slightly from those reported in the literature, which often includes joint pain, with ocular pain being less common. Therapy with albendazole showed to effective and better tolerated by patients.

## 274

### A GEOHELMINTH SURVEY OF INDIVIDUALS LIVING IN SIX DIFFERENT BRAZILIAN BIOMES: ITS IMPORTANCE IN THE NATIONAL MASS TREATMENT CONTROL PROGRAM

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Soil-borne helminthiasis, geohelminthiasis are infections caused by helminths that undergo part of their life cycle in the soil. The most prevalent are *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms that epidemiologically are typically linked to poverty and underdevelopment. In Brazil the existing data on these infections gives a poor picture of the actual epidemiological situation since it is based principally on old data from hospitals amd health posts. The present study was recommended by the Brazilian Health Ministry to obtain a more up-to-date vision of the epidemiology of geohelminthiasis in the country prior to a mass treatment campaign in association with OMS of 5 to 15 year olds from 719 municipalities. Its aim is to assess the frequency of soil-transmitted helminths in human fecal samples in the campaign´s target group and from co-inhabiting adults living in 35 municipalities belonging to six different biomes: Savana, Pantanal, Amazon Rainforest, Pampa, Caatinga and Atlantic Forest located in five Brazilian regions. The samples are collected from populations that are part of the Family Health Strategy (ESF's) or from basic health units or university hospitals. 200 samples are collected in each of the 35 municipalities totaling 7,000 fecal samples from six biomes. The samples are examined using the Hoffmann sedimentation techniques. Partial results (Pampa biome) show that of the 134 samples analyzed 48.5% were positive for some intestinal parasite. Of this total 26% were positive for helminths. Fecal samples of the Pantanal, part of the Amazon Rainforest and Caatinga are being analyzed. The remaining samples, including the Atlantic Forest and Cerrado will be collected during June and July 2013. The parasitological analysis of all 7,000 samples will be completed by October and the data will be presented in our poster.

## 275

### DEXAMETHASONE INHIBITS MICE BRAIN APOPTOSIS IN EOSINOPHILIC MENINGITIS CAUSED BY *ANGIOSTRONGYLUS CANTONENSIS* INFECTION

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*Angiostrongylus cantonensis*, also known as the rat lungworm, is the major cause of eosinophilic meningitis in the Pacific Islands and southeast Asia. Rats serve as the definitive host of the nematode. Humans are infected incidentally and lead to eosinophilic meningitis. Previous mice study had demonstrated increased apoptotic proteins and decreased anti-apoptotic proteins in mice infected with *A. cantonensis*. Steroids have been shown to be one of the effective treatment options for eosinophilic meningitis caused by *A. cantonensis* infection. However, the mechanism of how steroids can influence on eosinophilic meningitis are still unclear. We hypothesize that the beneficial effect of steroid on eosinophilic meningitis are mediated by decrease apoptosis. In a BALB/c mice model, mice were orally infected with 50 *A. cantonensis* L3 via an orogastric tube and were sacrificed every week for 3 consecutive weeks after infection until the end of the study. Dexamethasone was injected via the intra-peritoneal routine from 7th days of infection until the end of the study. Evans blue method was used to measure the blood brain barrier changes and the brain homogenates expression of apoptotic protein and anti-apoptotic protein were analyzed by western blot, immunohistochemistry and TUNEL assay. There was an increased Evans blue amounts, apoptotic proteins (caspase-3, 8, 9 and cytochrome C) and decreased anti-apoptotic proteins (bcl-2) expressions following 2-3 weeks of infection. Dexamethasone administration significantly decreased Evans blue extravasations and apoptotic protein expressions. In conclusion, apoptosis of mice brain homogenates can be repressed by treatment with dexamethasone.

## 276

### FACTORS ASSOCIATED WITH TIMELY IMPLEMENTATION OF MASS-DRUG ADMINISTRATION (MDA) FOR SOIL-TRANSMITTED HELMINTHIASIS (STH)

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More than one billion doses of medicine were shipped by pharmaceutical companies in 2012 for control or elimination of neglected tropical diseases (NTDs). This number is expected to increase to achieve 2020 targets established by the World Health Organization (WHO). Since 2007, Johnson & Johnson has donated Vermox® (mebendazole) for treatment of school-age children to control soil-transmitted helminthiasis (STH). National Ministries of Health or Education request Vermox through Children Without Worms (CWW), which has facilitated drug shipments and provided technical support. CWW reviewed program data on Vermox requests, production, shipments, and treatments for the 11 STH-endemic countries it supported during 2007-2011 to identify factors associated with on-time, delayed or missed mass-drug administration (MDA) campaigns. MDAs were categorized as on time (occurring in or before the planned month), delayed (occurring after but within 6 months of the scheduled month), or missed. Of 58 MDAs for which Vermox was requested and shipped (138,399,000 treatments), 42 (72%) were implemented on time, 8 (14%) were delayed, and 8 were missed. Of the 8 delayed MDAs, 7 occurred within one month of the scheduled date. In-country issues contributed to 6 (75%) of the delayed MDAs. Production, customs, and in-country challenges were some of the issues that contributed to missed MDAs, which occurred in 2008 and 2011, during periods of significant scaling up of the donation. Reported drug coverage rates were comparable between on-time and delayed MDAs (89% vs. 93%, $P > 0.9$). These data suggest that most MDAs are implemented on-time and that missed MDAs are more likely during periods of rapid program expansion. To avoid costs associated with delayed or missed MDAs, careful planning and collaboration are necessary among all stakeholders throughout the supply chain, from production to the community.

## 277

### WATER, SANITATION AND HYGIENE RISK FACTORS FOR SOIL-TRANSMITTED HELMINTH INFECTION IN NUEVA SANTA ROSA, GUATEMALA - 2010

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Soil-transmitted helminth (STH) infections cause significant physical and cognitive morbidity. Water, sanitation and hygiene (WASH) likely play roles in STH infection. We conducted a cross-sectional survey of a county in Guatemala to evaluate WASH risk factors for STH infection. We randomly selected households from roofs identified in aerial photos, then surveyed household members and performed environmental WASH evaluations using standardized questionnaires. We used WHO/UNICEF drinking water and sanitation ladders to classify household WASH infrastructure quality as improved or not improved. We tested stool from participants ≥ 1 year of age for STHs using the Mini Parasep® fecal parasite concentrator method and drinking water for *Escherichia coli* using the Colilert® most probable number (MPN) method. We performed unadjusted and multivariable analyses, the latter using the Lasso penalized regression shrinkage method to select variables. We used mixed models to account for household clustering. Models were weighted by the inverse number of roofs per household and adjusted for age category, socioeconomic status, and STH treatment in the past year. We tested 701 persons in 184 households for STH: 76 (11%) 1-4 year olds, 202 (29%) 5-14 year olds, 167 (24%) women of childbearing age 15-44 years old and 256 (37%) other adults ≥ lk15 years old. Most were female (56%), 215 (31%) reported deworming in the past year, and 88 (13%) were positive for *Ascaris*, *Trichuris* or hookworm. Drinking water available for <6 hours/day (prevalence ratio [PR] 4.06, P=0.002), crowding calculated as household members per bedroom (PR 1.24, P=0.049), finished floors (PR 0.25, P<0.001), improved drinking water (PR 0.28, P=0.050) and ever cleaning the sanitation facility (PR 0.33, P=0.026) were significant in multivariable modeling. Our findings support previous studies indicating STH infection is associated with WASH. Basic household interventions to improve safe drinking water availability and behavior changes concerning environmental cleanliness may be helpful in preventing future STH infections.

## 278

### TREAT WORM INFECTIONS WITH CRYSTAL PROTEIN EXPRESSING IN PROBIOTIC LIKE BACTERIA

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Soil-transmitted helminths (namely hookworms, whipworms, and *Ascaris* large roundworms) are intestinal nematodes, which cause diseases of poverty that infect upwards of two billion people worldwide. These parasites are a major threat to health and development of hundreds of millions of children and pregnant women. Enormous hurdles must be overcome in order to develop and deliver urgently needed new therapies

(anthelmintics) to replace old ones that perform sub-optimally and are losing efficacy. Any new therapy must be extremely cheap, be able to be produced in tremendous quantities to treat hundreds of millions of people, have a stable shelf life, and be capable of storage and delivery under adverse environmental conditions. Our research has uncovered a radical and unique new approach that solves each of these challenges: expression of vertebrate-safe, anthelmintic (anti-nematode) proteins in "probiotic-like" food-grade bacteria. Such bacteria can be produced cheaply, in great quantity, stored stably, and delivered under adverse conditions. Here we will discuss our work to develop such engineered bacterial therapy using the anthelmintic crystal protein Cry5B normally made by *Bacillus thuringiensis* (Bt). We will present data on how Cry5B can be expressed in a non-Bt bacterium related to food-grade bacteria, and the strong efficacy of such a bacterium in clearing hookworm infections in rodents. We will also update progress on engineering several food-grade bacteria to express Cry5B as a critical step towards implementation of this novel anthelmintic approach.

## 279

### A QUALITATIVE STUDY EXPLORING BARRIERS RELATED TO USE OF FOOTWEAR IN RURAL HIGHLAND ETHIOPIA: IMPLICATIONS FOR NEGLECTED TROPICAL DISEASE CONTROL

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The role of footwear in protection against a range of Neglected Tropical Diseases (NTDs) is gaining increasing attention Better understanding of the behaviors that influence use of footwear will lead to improved ability to measure shoe use and will be important for those implementing footwear programs. Using the PRECEDE-PROCEED model we assessed social, behavioral, environmental, educational and ecological issues and needs influencing whether and when children wear shoes in a rural highland Ethiopian community endemic for podoconiosis. Information was gathered from 242 respondents using focus groups, semi-structured interviews and extended case studies. Shoe-wearing norms were said to be changing, with going barefoot increasingly seen as 'shameful'. Shoes were thought to confer dignity as well as protection against injury and cold. However, many practical and social barriers prevented the desire to wear shoes from being translated into practice. Limited financial resources meant that people were neither able to purchase more than one pair of shoes to ensure their longevity nor afford shoes of the preferred quality. As a result of this limited access, shoes were typically preserved for special occasions and might not be provided for children until they reached a certain age. While some barriers (for example fit of shoe and fear of labeling through use of a certain type of shoe) may be applicable only to certain diseases, underlying structural level barriers related to poverty (for example price, quality, unsuitability for daily activities and low risk perception) are likely to be relevant to a range of NTDs. Using well established conceptual models of health behavior adoption, we identified several barriers to shoe wearing that are amenable to intervention and which we anticipate will be of benefit to those considering NTD prevention through shoe distribution.

## 280

### INDIVIDUAL CORRELATES OF PODOCONIOSIS IN AREAS OF VARYING ENDEMICITY: A CASE-CONTROL STUDY

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Podoconiosis is a form of non-filarial elephantiasis resulting in bilateral and usually asymmetric lymphedema of the lower legs limited to below the knees. It is common among barefoot people who live in highlands surfaced by red clay soils. In the present study, we aimed to understand the individual correlates associated with podoconiosis by comparing podoconiosis-affected cases and unaffected controls living in areas with three levels of podoconiosis prevalence: 'low' (< 1%), 'medium' (1-5%) and 'high' endemicity (> 5%). Cases (n = 460) and controls (n =707) were recruited from six *kebeles* (the lowest administrative unit) in northern Ethiopia. Data were collected by trained community health workers that identified cases going house-to-house and then by nurses who interviewed identified cases and controls. Cases, especially women, were less educated (OR = 1.73, 95% CI = 1.34 to 2.23, $p < 0.0001$), less likely to be married (OR = 3.43, 95% CI = 2.57- 4.57, $p < 0.0001$) and had lower income ($t = -4.39$, $p < 0.0001$) than controls. There was no statistically significant difference between controls residing in the three areas with varying levels of podoconiosis endemicity. Even though there were no significant differences in foot-washing practices, study subjects with dirty feet were twice (OR = 1.85, 95% CI = 1.36 to 2.51, $p < 0.0001$) as likely to be cases, and those with cracked feet four times (OR = 4.18, 95% CI = 2.738 to 6.370, $p < 0.0001$) more likely to be cases than controls. On average, cases started wearing shoes ten years later than controls ($t = 8.15$, $p < 0.0001$). Among cases, age at first wearing shoes exhibited a positive correlation with age of onset of podoconiosis ($r = 0.63$, $t = 12.51$, $p < 0.0001$). Among all study participants, age at first wearing shoes showed a strong positive correlation with age ($r = 0.81$, $p < 0.001$), and average duration of shoe wearing was less than 30 years, indicating secular increases in shoe wearing over recent years. There was clustering of podoconiosis cases within households, with 142 (30.7%) households containing two or more cases. Based on these findings, we recommend that interventions against podoconiosis adopt household-targeted case tracing. In addition, efforts should be made to bring forward onset of shoe-wearing and to address inequalities in education, income and marriage opportunities, particularly among women.

## 281

### EXTENDING HELMINTH CONTROL BEYOND STH AND SCHISTOSOMIASIS: THE CASE OF HUMAN HYMENOLEPIASIS

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Hymenolepiasis caused by the cyclophyllidean tapeworm *Hymenolepis nana* is the most prevalent human cestodiasis in the world. Similarly to urogenital schistosomiasis, *H. nana* infections can be treated with praziquantel but endemic areas may not overlap. To observe the need to deliver praziquantel to treat *H. nana* infections and investigate the level of overlap with *S. haematobium* infections we used data on *H. nana* infection in children aged ≤15 years collected during an epidemiological survey carried out in northern Angola from May to August 2010. We found that poor sanitary conditions of communities are an important contributor to the high prevalence of *H. nana* infection in the study area. We have also shown that *H. nana* infection is an important contributor to morbidity,

particularly in children aged <5 years. Importantly, our results demonstrate that the spatial distribution of urogenital schistosomiasis and *H. nana* infections differ and distributing praziquantel solely to treat urogenital schistosomiasis will overlook areas with *H. nana* infections. Given the ubiquity and morbidity effects of *H. nana* infections this represents a significant praziquantel gap and advocacy for including this infection in the list of neglected tropical diseases could constitute an important first step towards acknowledging its epidemiological importance.

## 282

### QUANTIFYING THE EXTENT OF MALARIA AND ONCHOCERCIASIS CONTROL ACTIVITIES IN RELATION TO LYMPHATIC FILARIASIS ELIMINATION: A MULTIPLE INTERVENTION SCORE MAP (MISM)

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Lymphatic filariasis (LF) is being targeted for global elimination by 2020, primarily by interrupting transmission through annual mass drug administration (MDA) with either ivermectin or DEC, plus albendazole to the population at risk for at least five consecutive years. Many countries are making good progress, however there is need to better quantify the different levels of MDA coverage, plus other interventions such as ivermectin for onchocerciasis, and vector control for malaria, to determine if high coverage rates of multiple interventions sufficiently overlap high prevalence areas. This study aimed to develop a measure for multiple intervention coverage at both district and sub-district levels. Using Malawi as a case study, we obtained data on MDA coverage (health centre level) from the National LF elimination programme, ivermectin distributions (district level) from onchocerciasis reports, bed net coverage (community level) from multiple Demographic and Health Surveys and indoor residual spraying (IRS) from published literature. Using spatial interpolation methods, coverage levels for MDA and bed nets were smoothed to derive spatially continuous estimates of these measures. Each coverage measure was classified into four levels e.g. for MDA the levels were 0-40%, 40-65%, 65-80%, >80%. Each class was assigned a value between 0 (low) and 3 (high), and these values were summed to produce a single score for each intervention. These intervention scores were combined in a weighted sum, where weights depended on the relative importance of the intervention, and a multiple intervention score map (MISM) was produced. This map highlighted areas in the far north and along the coast of Lake Malawi where consistently high bed net coverage plus IRS may have significantly impacted and/or potentially eliminated LF prevalence, despite lower than average MDA coverage. This multiple intervention score methodology can be easily applied to data from other LF endemic countries, and help guide national LF programmes' future intervention efforts.

## 283

### COMMUNITY PARTICIPATION (CP) ASSESSMENT OF A SCHISTOSOMIASIS CONTROL PROGRAM IN A RURAL AREA OF MINAS GERAIS STATE, BRAZIL

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A schistosomiasis control study was developed using the CP approach in São Pedro a rural community in the municipality of Jequitinhonha Brazil. A modified Rifkin et al. (1988) spidergram was developed for use in São Pedro to measure the extent of CP in five dimensions: 1) leadership of the community and professionals introducing the intervention, 2) planning and management partnerships between the community and professionals, 3) communication, 4) external support, and 5) monitoring and evaluation (M&E) of the participation of intended beneficiaries. Forty-one community leaders and representatives, including local health professionals and district government managers of construction/infrastructure/transportation and municipal health offices participated in the project. The program was developed in three phases, with six meetings each, involving local and district participants and researchers. The participants agreed to improve the water supply/sanitation and build a laboratory (district government managers), attend community meetings, develop a local health community council (community representatives), and provide health education, diagnostic and schistosomiasis treatment services (researchers). All designated actions except the construction of a laboratory and a sewage treatment system were completed. Preliminary spidergram assessment indicates that whereas CP in leadership, planning and management, and community was satisfactory, scoring 3 each on a five point scale, external support and M&E lagged behind (scoring 2 points each). Moreover, while attendance of meetings was overall 60%, it declined after initial high attendance rates and male participation was low throughout the two-years of the project. The results indicate that the spidergram method can measure CP in schistosomiasis prevention and control. However, the program needs to strengthen community capacity to sustain the intervention, improve feedback to the community and facilitate participation in data collection and M&E.

## 284

### HOW THE PRIVATE SECTOR HAS MADE A DIFFERENCE IN NEGLECTED TROPICAL DISEASES CONTROL PROGRAMS

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The fact that the term 'neglected tropical diseases' (NTDs) was first used in 2004/2005 to describe parasitic and bacterial diseases that affected the poorest of the poor, and that the global movement to reduce the burden of these diseases has significantly intensified with multimillion dollar grants, illustrates that the global health community has prioritized the need to control these diseases. The movement significantly intensified in January 2012 when the London Declaration announced unprecedented commitments to support the WHO's goal of controlling and eliminating 10 of the NTDs by 2020. Even as the global movement to end NTDs continues to gain momentum, these diseases continue to inflict suffering and chronic disability on 1.5 billion of the world's most impoverished. In an effort to mobilize the necessary funding to expand control efforts and reach the 2020 NTD goals, the private sector has actively intensified its support. The END Fund, the world's first private initiative aimed at tackling NTD was launched in June 2012. Since launching, the END Fund has progressively expanded support efforts and is now working in 13 countries. When Mali's funding from USAID was frozen due to the 2012 coup d'etat, the END Fund was able to mobilize the necessary US$1.2m to re-launch the country's mass treatment campaign. By identifying a group of private sector donors, including gold mining companies and private foundations, the END Fund ensured Mali's NTD control program did not lose the impressive gains made in the previous years to reduce the NTD burden throughout the country. The END Fund and its private sector investors are committed to improving the health of those affected by NTDs. By establishing multidisciplinary partnerships, the END Fund brings new donors to the NTD movement and invests the much needed funding into control programs. By helping to treat communities and improving the health and well being of societies as a whole, the END Fund is contributing to breaking the poverty cycle that NTDs force upon the world's most impoverished.

## 285

### DOES CLINICAL ACTIVITY PREDICT CHLAMYDIAL INFECTION AFTER MASS ANTIBIOTIC TREATMENTS FOR TRACHOMA?

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Global efforts to eliminate blinding trachoma target the ocular strains of *Chlamydia* trachomatis that cause the disease. Mass antibiotic distributions are a major strategy for trachoma elimination. Testing for chlamydial infection by PCR is expensive, so clinical examination is widely used to determine whether or not mass antibiotic distributions are necessary. Previous studies reveal a relatively high correlation between clinical activity (TF or TI utilizing the WHO grading system) and chlamydial infection before mass treatment. Unfortunately, this correlation decreases dramatically after mass treatment--it is difficult to determine who is infected by clinical exam after treatment. However, treatment is at the community level. Here, we assess whether the prevalence of clinically active trachoma predicts chlamydial infection at the community level, using longitudinal results from a cluster-randomized controlled trial of mass azithromycin distributions in Niger. 48 Nigerien communities were randomized to annual or biannual treatment. Every six months, a random sample of up to 100 children (0 - 5 years of age) in each of the 48 communities were examined for trachoma, and conjunctival swabs were collected for chlamydial testing with PCR. The mean antibiotic coverage was 88.2% per community, with a mean of 89 children examined and 168 children treated. At baseline, the correlation between the prevalence of clinical trachoma and chlamydial infection was 0.64 (95% CI: 0.38 to 0.79) and by 24 months, 0.47 (95% CI: 0.23 to 0.70). At 24 months, we were unable to demonstrate that the correlation coefficient was lower than that at baseline (p = 0.42). Most trachoma elimination programs utilize clinical examination to determine their mass antibiotic strategy due to the high costs and resources associated with PCR processing to test for chlamydial infection. While clinical exam has not been shown to be useful at the individual level, this study suggests that clinical activity and chlamydial infection are correlated, even after mass antibiotic treatment. We were unable to find a significant difference in the correlation at baseline and 24 months.

## 286

### ERADICATION INVESTMENT CASE FOR ONCHOCERCIASIS

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The health and economic benefits of disease eradication may be substantial, but costs and consequences should be carefully evaluated. An Eradication Investment Case (EIC) is an analytic-deliberative process to support decisions on whether to launch eradication initiatives. The EIC for onchocerciasis was developed adopting an approach, proposed at the Ernst Strüngemann Forum 2010, including feasibility assessment, the estimates of costs, and the health and economic impacts. Strategies to achieve elimination and eradication were developed as scenarios. The number of treatments, the financial and economic costs of delivering them, and the public health and economic impacts were assessed for the time horizon 2013-2040. Costs were estimated using a micro-costing approach, while the public health impact was estimated with a micro simulation model. Geographical scaling up of treatment coverage to achieve elimination in African countries would require 48 million treatments annually on average, less than a half of the annual treatments needed, if current control levels are maintained through 2040. Accordingly, the annual financial cost for 2013-2040 will decrease from $23 million to $10 million as the goal shifts from control to elimination or eradication. Scaling up and sustaining the treatment coverage for onchocerciasis elimination and eradication is also predicted to lead to substantial economic benefits in the long run, which are only marginally reduced by the increase in surveillance costs to sustain elimination, mainly due to direct and indirect cost savings of averting skin-itch cases. Marginal health benefits of onchocerciasis elimination versus control are more limited, since the current control strategies already reached high treatment coverage in areas where onchocerciasis is considered to be a public health problem; and non-covered areas mainly concern low endemic areas. The EICs shows that a goal shift to elimination and eradication of onchocerciasis is predicted to generate substantial future economic benefits.

## 287

### MEDICAL STUDENT KNOWLEDGE OF NEGLECTED TROPICAL DISEASES IN PERU

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Education to health-care professionals is a cornerstone in the battle against neglected tropical diseases (NTD) in developing countries. Few studies have evaluated the level of knowledge of undergraduate medical students in clinical and epidemiological aspects of NTD. The aim of this study was to describe the level of knowledge in NTD among medical students from a private school of medicine in Lima, Peru. A twenty-multiple choice questionnaire was given to students from first to seventh year (last year) of medical school of Universidad Peruana Cayetano Heredia in Lima, Peru; to be completed confidentially in 10 minutes. The questionnaire was composed by two blocks of questions. Block I consisted of epidemiological questions and block II of clinical vignettes. We arbitrarily defined knowledge as low or suboptimal (≤ 30% correct answers), moderate or acceptable (31-69%) and high or outstanding (≥70%). A total of 586 students (49.3% female, 50.7% male; age mean ± SD = 21.3 ± 2.4 years) completed the voluntary and anonymous survey. The prevalence of high-score grades of knowledge increased significantly from first-year (1.9%) to senior medical students (57.9%) (p=0.0001). Higher outstanding scores were found in clinical (85.9%) compared to epidemiological knowledge (21%) in last-year medical students (p=0.0001). In contrast, lower scores (suboptimal) were more prevalent in epidemiological (24%) than clinical knowledge (5.3%) in senior medical students (p=0.01), which indicates that clinical knowledge improves towards the end of career whereas epidemiological concepts remain low. In conclusion, students from this medical school in Peru have an outstanding knowledge about NTD at the end of the career mainly in clinical concepts, but acceptable or suboptimal in the epidemiological area. These results stress the importance of intensifying the education in epidemiology of NTD in undergraduate medical students at developing countries.

## 288

### LARGE-SCALE INTEGRATION OF PUBLIC HEALTH ACTIVITIES FOR TROPICAL DISEASES IN TOGO

**Kodzo A. Anthony**[1], Kokou-Sika Dogbe[2], Mouawiyatou Bouraima[2], Kokou Tossa[2], Pabanam N'Zonou[3], Potchoziou Karabou[2], Stephanie A. Richard[1], Rachel Bronzan[1], Anders Seim[1], Koffi S. Sognikin[2]

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Togo, a country in west Africa, is endemic for several neglected tropical diseases, including onchocerciasis, schistosomiasis, and soil-transmitted helminthes (STH). Togo has been a pioneer in integrating public health activities for tropical diseases, beginning as early as 2004. The first nationwide mass drug administration (MDA) occurred in August 2011, and included not only treatment for onchocerciasis, schistosomiasis, and STH for persons five years old and older, but also vitamin A and STH treatment for children under 5 and insecticide treated bed net distribution for each household. The 2011 integrated MDA involved many partners, including different sections of the Togo Ministry of Health as well as external partners including the Global Fund, USAID, Health and Development International, UNICEF, Red Cross, Vestergaard, World Health Organization, Plan Togo, OCDI, and Sightsavers. The MDA was implemented by over 5,600 community drug distributors and took place in three phases. The first phase involved a census, vitamin A supplementation, albendazole treatment based on local STH prevalence, and ivermectin treatment based on local onchocerciasis prevalence. The second phase included distribution of coupons for insecticide treated bed nets for each household and administration of praziquantel based on local schistosomiasis prevalence. The third phase of the 2011 MDA involved distribution of the bed nets. Ultimately, the integrated MDA of 2011 treated 1.3 million children for schistosomiasis, 1.8 million children for soil-transmitted helminthes, and 2.4 million people for onchocerciasis, supplemented over 900,000 children with Vitamin A, and distributed over 2.5 million bed nets. These activities were possible in 2011 only through the efficiency of large-scale integration across programs. Integrated activities, however, are more complex and require greater coordination at all levels of the health system in order to succeed. The strong leadership at the Ministry of Health promoted an unprecedented degree of partnership and sharing of resources among the different organizations, making this integrated campaign a success.

## 289

### FACTORS UNDERLYING THE SUCCESSFUL LARGE-SCALE INTEGRATION OF PUBLIC HEALTH ACTIVITIES FOR TROPICAL DISEASES IN TOGO

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Togo has a long history of integrated health activities for control of tropical diseases, beginning as early as 2004. The first nationwide mass-drug administration occurred in 2011, and included not only integrated treatment of onchocerciasis, schistosomiasis and soil-transmitted helminthes, but also vitamin A supplementation and insecticide treated bed net distribution for each household. This large-scale integration of public health activities was made possible by strong leadership from the Togo Ministry of Health (MOH), which coordinated the shared training, logistical, and supervisory activities. Four committees, each of which included representatives from each partner organization, organized the integrated activities. The Technical Committee developed the shared training materials and reporting tools and organized successive trainings of the district-level supervisors, followed by the sub-district-level supervisors, and finally the community drug distributors. The Logistics Committee was responsible for ensuring all of the training materials, reporting forms, registers, bed net coupons, drugs, vitamin A, and insecticide-treated bed nets were distributed across the country. The Social Mobilization Committee was responsible for informing the public about the upcoming integrated public health activities and generating public service announcements. Finally, the Finance Committee was in charge of the integrated budgets and ensuring that there were sufficient resources for all of the integrated activities. Although many challenges were faced, the Togo MOH managed to lead all of the Committees to a successful integrated activity in August 2011. Overall, the integration of albendazole, ivermectin, and praziquantel treatment, vitamin A supplementation, and bed net distribution demonstrated the efficiency of an integrated approach. Each of the partners contributed to the shared activities, which resulted in more people receiving greater public health benefits than would have otherwise been possible.

## 290

### INTERACTIONS BETWEEN COENZYME Q10, MELARSOPROL AND *TRYPANOSOMA BRUCEI RHODESIENSE* INFECTION MODULATE ANTI-OXIDANT DYNAMICS MOUSE MODEL

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Melarsoprol (Mel B) is the only efficacious drug against Late stage Human African Trypanosomiasis (HAT), but inadvertently induces Post Treatment Reactive Encephalopathy (PTRE) and unacceptable mortality of 5% among patients. Investigations were conducted to establish neuro-protective role of specific antioxidants (Manganese Superoxide dismutase (MnSOD), Glutathione Reductase (GR), Copper-Zinc Superoxide dismutase (SOD-1) and reduced glutathione (GSH) and Coenzyme Q10 CoQ10) against PTRE and putative resultant brain degeneration in a mouse model. Additionally, the role of antioxidant capacity in neurodegeneration due to *Trypanosoma brucei rhodesiense* was investigated. Female Swiss-white mice were infected with *T. brucei rhodesiense* parasite and were manipulated to simulate all phases of HAT and PTRE. Expression profiles of the antioxidants in brain tissues were assessed using immunoblot or spectrophotometric procedures. There were significantly higher expressions of MnSOD (P=0.0014), SOD-1 (P=0.0001), and GR (P=0.0083) in infected than uninfected mice 21 days post infection (dpi), which were two-folds lower than those observed at 57 dpi. Levels of GSH were significantly lower (P=0.0347) in infected 57dpi than uninfected mice 21dpi. Expressions of SOD (P=0.0429), GR (P=0.0001) and GSH (P=0.0001) were significantly higher in infected than in uninfected mice among Mel B treated mice. Mel B treated uninfected mice had significantly lower expressions of GR (P=0.0001) and GSH (P=0.0001) and MnSOD (P=0.0035), than untreated (uninfected) controls. Pre-treatment with CoQ10 significantly increased expression of GSH (P=0.0001) relative to the Mel B treatment alone. The results indicate that the parasites and Mel B suppress the antioxidant system, while CoQ10 appears to restore it. The time dependent dynamics of antioxidant suppression due to Mel B, and potential ameliorating effects of CoQ10 on the same, indicate putative mechanism underlying and antidote to the toxicity of the drug with potential application in formulation of novel Mel B based drugs and development of novel markers for staging the disease.

## 291

### PROTEOMIC APPROACH FOR THE IDENTIFICATION OF NOVEL MARKERS FOR THE DIAGNOSIS OF VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL) is an infection transmitted by phlebotomine flies and caused by parasite of the genus *Leishmania*. This disease is characterised by fever, weight loss, enlargement of the liver, spleen and

lymph nodes and low blood cell count. Early and accurate laboratory diagnosis is essential before initiating treatment, for clinical outcome reasons. The clinical features of VL resembles those of several other disease including malaria, tuberculosis etc, effective drug are available but they need to be administered for a minimum 3 weeks and are potentially toxic and expensive. For diagnosis of VL, rK39 antigen based rapid test is widely used. Unfortunately, up to 32 % healthy individuals from endemic region test positive with this antigen in Indian subcontinent. There is an urgent need to search for a more specific antigen with more precise specificity but sensitivity similar to rK39 antigen. We identified *Leishmania donovani* specific 70kDa (BHUP1), 37kDa (BHUP2), 12.6kDa (BHUP3) soluble promastigote antigen through western blot technique, in order to develop a diagnostic marker for VL. On blotting, antibody against this protein was recognized by all VL patient's sera, but, it was absent in every control group non-endemic healthy control (NEHC), endemic healthy control (EHC) and different disease (DD). The diagnostic potential was further validated by ELISA using serum of VL, NEHC, EHC and DDs. The Sensitivity of the ELISA were found to be for VL (96, 95, 88%), whereas the specificity ranges for EHC (96, 98 and 96 %), NEHC (100% for three antigen) and DD (97, 97 and 97.4 %) groups respectively, against BHUP1, BHUP2, BHUP3 antigen respectively. Furthermore, it was characterized by 2D-PAGE analysis followed by MALDI-TOF analysis. Due to excellent sensitivity and specificity of these antigens, it warrants the further development as a tool for diagnosis of VL.

## 292

### HIGH-THROUGHPUT IN VITRO SCREENING OF SELECTED GHANAIAN MEDICINAL PLANTS FOR TRYPANOCIDAL ACTIVITY AGAINST T. BRUCEI

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Human African Trypanosomiasis (HAT) is a devastating disease in Africa. Available drugs are characterized with unpleasant limitations. This has necessitated the development of alternate treatment for trypanosomiasis. Ghana has a long history on use of traditional medicines which have not been scientifically evaluated. This is needed to establish any as new chemotherapy for trypanosomiasis. Thus, our aim was to establish a high-throughput in vitro screening system for trypanocidal drug candidates among selected Ghanaian medicinal plants (GMP). Crude extracts from selected GMP were screened for trypanocidal activity against the GUTat 3.1 strain of T. brucei using a 3-step in vitro system. Effects of the extracts on T. brucei viability and proliferation were determined by Alamar blue® assay (Invitrogen). Subsequently parasites were subjected to FACS analysis to investigate extracts' ability to induce apoptosis and/or cell cycle alteration. Finally, immunohistochemistry was used to detect any extract-induced morphological and marker expression changes in parasites. Crude extracts with activity in the first round 3-step screening were fractionated and re-screened. Further purification of active fractions into compounds was then done. The purified compounds were also taken through this 3-step screening for identification of active compounds. Out of 113 crude extracts screened, 8 showed strong trypanocidal activities and induced apoptosis. In addition, some extracts showed G2/M phase alteration during cell cycle in trypanosomes. Upon further analysis, a total of 4 including 2 novel active compounds have been identified. These caused flagellum deficiency in the parasites. This 3-step screening system enabled us to follow the interesting molecular activities of active components at various fractionation and purification stages. In vivo studies for efficacy and safety of the active compounds are ongoing. Our high throughput screening system successfully identified molecular mechanisms such as apoptosis and cell cycle alterations in the parasites.

## 293

### MOLECULAR TARGETS AND ANTILEISHMANIAL DRUG LEADS IDENTIFIED THROUGH SCREENING A LIBRARY OF NATURAL PRODUCTS PREPARED BY HIGH THROUGHPUT FRACTIONATION PARADIGM

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Visceral leishmaniasis, caused due to infection of *Leishmania donovani*, is a prominent disease in certain tropical regions of the world. Toxicity and suboptimal efficacy of current antileishmanial drugs necessitate the discovery of new antileishmanial drugs with better efficacy and safety profiles. Natural products remain an unmatched source of drugs with novel chemotypes. However conventional screening of crude natural products extracts suffers from certain limitations. Polyphenols, can cause false-positive results in *in vitro* screening. The presence of chemical diversity found a single extract. Biologically active compounds present at extremely low concentrations in crude extracts. Pre-fractionation technologies have been useful in overcoming these limitations. A library of >30,000 natural product fractions have been generated through a high throughput fractionation paradigm. Each fractions, in 96 well plates, have been , analyzed via QC-UPLC MS/MS. 7387 fractions from 671 plant extracts were screened through newly developed *L. donovani* Macrophage (THP1 cells) amastigotes parasite rescue and transformation assay and for cytotoxicity against differentiated THP1 cells. A total of 194 fractions were identified with 50% or higher inhibition vs. leishmania amastigote growth in THP1 cells; only 10 of these were toxic against the THP1 cells alone. The active 194 fractions were further screened for dose-response antileishmanial analysis. The selective antileishmanial activity of 65 fractions was confirmed. Active fractions from *Thuja occidentalis* (IC50-0.25 mg/mL), *Asclepias asperula* ($IC_{50}$-0.33 mg/mL), *Rhodea japonica* ($IC_{50}$-0.41mg/mL) and *Nerium oleander* ($IC_{50}$-0.03 mg/mL) were selected for further investigations. Deoxypodophyllotoxin from *T. occidentalis*, oleandrin from *N. oleander*, rhodexoside from *R, japonica* and a cardinolide from *A. asperula* were identified as actives, and potential new antileishmanial drug leads. These diverse compounds, which are potential inhibitors of DNA Topiosmerases and Na+,K+-ATPases, were selectively active against intracellular amastigotes with so significant effects on leishmania promastigotes. The results indicate Na,K-ATPase as potential new molecular drug target and confirm topisomerase as the promising target for new antileishmanial drug discovery.

## 294

### EFFECT OF FE, ZN AND CU IN VITRO PRODUCTION OF IFN-Γ, IL-13 BY PERIPHERAL BLOOD MONONUCLEAR CELLS IN PATIENTS WITH CUTANEOUS LEISHMANIASIS WITH TREATMENT FAILURE IN BOLIVIA

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Resistance to pentavalent antimonial(SBV) agents as Glucantime, is creating a major problem in the treatment of cutaneous leishmaniasis (CL). Generate new therapeutic approaches in the treatment regimen that could reverse antimony resistance is a public health problem in Bolivia. An appropriate balance between pro-inflammatory and anti-inflammatory cytokines that mediate innate and adaptative immune responses is required for effective protection against human leishmaniasis.

The study evaluated the effect of Fe, Zn and Cu as an alternative in the specific treatment and its influence on the immune balance in patients with CL with therapeutic failure (RESISTANT) and patients who responded successfully to treatment (SENSITIVE) enrolled as cases-controls respectively. Peripheral blood mononuclear cells (PBMC) were stimulated *in vitro* with soluble antigen proteins of leishmania *sp.*, to determine T-cells responses in cell culture medium supplemented with Fe, Zn and Cu. Anti *Leishmania* humoral and cellular immune response was evaluated by: The production of INF-γ and IL-13, as markers of Th1 and Th2 response respectively. Cellular response to the superantigen SEB were also monitored in this study. The data obtained indicate that PBMC of sensitive and resistant patients displayed an increased production of IFN-γ in absence of these trace elements that are associated with the low production of IFN-γ. This was associated with a type 1-biased immune environment, since cells of Resistant and Sensitive groups produced higher INF-γ levels in response to SEB. Resistant patients produce more IFN-γ in response to leishmania Ag that sensitive patients. IL-13 production remained low and similar in the two study groups, whatever the condition of stimulation. Our data indicated that these trace elements may influence the balance of TH1 cytokine production and possible therapeutic administration in parallel with the specific treatment should be evaluated, since the production of IFN-γ significantly decrease by effect of Fe, Zn and Cu and might augment susceptibility to infection.

## 295

### PARASITE PERSISTANCE AND CLINICAL RESPONSE TO MILTEFOSINE IN PATIENTS WITH CUTANEOUS LEISHMANIASIS

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Miltefosine (MLF) is an oral drug approved for the treatment of leishmaniasis in adults and children >2 years of age. Knowledge of parasitic response to treatment with miltefosine is limited. Previous studies have demonstrated persistence of parasites in patients with treatment failure and case reports have shown decrease in parasite load after treatment in patients with clinical cure.This study aimed to determine the parasitologic response to MLF during and after treatment of cutaneous leishmaniasis caused by species of the *Viannia* subgenus, and its relationship with the therapeutic outcome. A sample size of sixty was estimated for this study based on population pharmacokinetic modeling. Participants received supervised treatment with MLF (1.8-2.5 mg/kg/day, during 28 days). Clinical response was evaluated at the end of treatment, and 13 and 26 weeks after initiation of treatment. Presence of *Leishmania* was determined by kDNA PCR in aspirates and swabs of lesions/scars obtained pre-treatment, days 15 and 29, and 13 weeks after beginning treatment. To date 49 patients, 22 children (2 - 12 years old) and 27 adults (18-60 years old) have been enrolled; 26 patients have completed follow up with a final cure rate of 100% (n=15) in adults and 81.8% (n=11) in children. Apparent cure was observed in 6.8% (3/44) at the end of treatment and in 54% (20/37) at week 13. Pre-treatment lesion samples from all patients were positive for kDNA. At day 15, 27% (10/36) and post-treatment 23% (9/39) of patients were kDNA positive. Thirty percent (6/20) patients with apparent cure at week 13 were kDNA positive. In conclusion, these initial observations confirm the persistence of parasites following clinical cure in response to miltefosine treatment, and raise concerns regarding the potential for emergence of drug tolerant populations.

## 296

### TREATMENT OF BOLIVIAN CUTANEOUS LEISHMANIASIS WITH INTRALESIONAL DRUGS

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Cutaneous leishmaniasis (CL) is a disease that ultimately self-cures. Whether local or systemic therapy should be used to treat this disease is presently undecided. Factors favoring systemic therapy are high cure rate and theoreticaly protection against mucosal dissemination. Factors favoring local therapy are the presence of only 1 lesion, the rarity of mucosal dissemination which systemic CL therapy might prevent, and adverse effects of systemic drugs. We have reported a 6-month cure rate of 70% in 30 patients with single Bolivian CL lesions treated with 3 intralesional injections of pentavalent antimony. When failure did occur, it was seen at the 1 month and 3 month follow up periods. There was no relapse at 6 months of previously cured disease. Adverse effects were limited to mild injection site pain. In comparison, a placebo group of 30 patients demonstrated a 17% cure rate. A cryotherapy group of 20 patients had a surprisingly low cure rate of 20%. To attempt to increase the cure rate yet not overly burden patients with clinic visits, we are now evaluating 5 intralesional injections of pentavalent antimony and also intralesional injection of other antileishmanial agents. Efficacy at intermediate and 6 month follow up periods in the present study will be presented in comparison to that of the active and placebo groups of the prior study. The possible adverse effects of pain, itching, inflammation, and vesicular formation in the present study will also be presented in comparison to the values for the active and placebo groups of the prior study. An overall evaluation of efficacy, tolerance, and feasibility (a particular issue for repeated physical interventions such as topical application of drugs) for local measures vs systemic treatments will be presented.

## 297

### A RETROSPECTIVE ANALYSIS OF 400 CASES TREATED FOR VISCERAL LEISHMANIASIS IN GEORGIA

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Visceral Leishmaniasis (VL) is one of the most widespread zoonotic infections in Georgia. The causative agent of VL is L. infantum. It mostly affects children under 5 years old. In recent years, the number of new cases among adult population has increased. HIV/Leishmaniasis co-infection has also increased during the last several years, which is characterized with increased resistance to treatment and high rates of relapse. We studied the medical records of 400 patients with visceral Leishmaniasis who were diagnosed and treated between 2009 and 2012. Among these 400 patients, 300 were treated with Meglumine Antimoniate (Glucantim) and 100 were treated with Lyposomal Amphotericin B (Ambisome). The dosage of Glucantime constituted 20 mg/kg and summary dosage of Ambisome constituted 20-30 mg/kg. Among those treated with Glucantim 292 (97.3%) patients were cured and in 8 (2.7%) patients the treatment was stopped. Among these 8 patients 5 died. In 3 cases the cause of death was liver failure and in 2 cases severe arrhythmia due to toxic side effects of Glucantime. In the rest of patients Glucantime treatment was stopped due to drug induced liver failure or cardiac arrhythmia. Side effects of Glucantim treatment was observed in 39 (13%) patients. Relapse after Glucantim treatment

was observed in 14 (4.6%) cases. Among those who were treated with Ambisome (100 patients) complete course of treatment was received by 97 (97%) of patients and in 3 (3%) cases the treatment was interrupted because of lethal outcome in 2 cases and allergic reaction in 1 case. Side effects of Ambisome treatment was observed in 7 (7%) patients. Among 12 study participants who had HIV/*Leishmania* co-infection 9 (75%) patients developed relapse and 3 (25%) patients died. Relapse occurred after the treatment both with Glucantime and Ambisome. All HIV infected study participants had more than 2 co-infections and CD4+ count with < 200 cell/μL. Relapse occurred even after increasing the dose of Ambisome to 30 mg. Both anti-leishmania drugs (Glucantime and Ambisome) manifested the high clinical effectiveness. The effectiveness of both drugs in patients with HIV/*Leishmania* co-infection was very low.

## 298

### *IN VITRO* SCREENING OF COMPOUNDS AGAINST *LEISHMANIA AMAZONENSIS* AMASTIGOTES

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There is an estimated burden of 12 million infections by *Leishmania* worldwide. Without an effective vaccine and few drugs, there is a need for new treatment alternatives. Despite advances in parasite biology and genetics, development of new compounds is limited. High Throughput Screening of compounds with methods closely mimicking natural mammalian infection brings advantages to new drug development. We performed a pilot *in vitro* screen of 2400 compounds at 10 μM using the DIVERSet Library (Chembridge Corporation) on wild-type *L. amazonensis* axenic amastigotes measuring cell viability via alamarBlue® fluorescence. Selecting hits with at least 75% activity with respect to Pentamidine control yielded 53 compounds (2.2%). The assay had good signal to background ratio, with a five-fold difference between controls and Z-scores ranging from 0.52 to 0.63. Hits were then tested for efficacy against infected J774 macrophages with a strain of *L. amazonensis* expressing β-lactamase. Ten compounds (18%) had significant activity against intra-macrophage amastigotes at 10 μM, but with lower efficacy compared to axenic amastigotes. J774 cell toxicity curves showed that 22% of compounds were not toxic at a concentration of 200 μM, 55% were toxic at 200 μM but had no effect at 10 μM while 23% had toxicity below 10 μM. Thus, we have selected 6 hits with high intra-macrophage efficacy and low toxicity to host cells. The results obtained from this pilot screening assay with *L. amazonensis* amastigotes show good reproducibility and reliability, validating this assay for high throughput screening. Furthermore, the intracellular and toxicity assays enable a better narrowing down of compounds for future *in vivo* assays to assess clinical efficacy.

## 300

### STUDIES ON THE MECHANISM OF ACTION OF THE ARYLIMIDAMIDE DB766 IN *LEISHMANIA DONOVANI*: ROLE OF CYP5122A1 AND AZOLE INTERACTIONS

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Arylimidamides (AIAs) show potent activity against intracellular parasites. The frontrunner AIA DB766 (2,5-bis[2-(2-*i*-propoxy)-4-(2-pyridylimino) aminophenyl]furan hydrochloride) displays potency similar to amphotericin B against intracellular *Leishmania* amastigotes and is orally active in animal models of visceral leishmaniasis, but its mechanism of action is unknown. Ultrastructural studies in *L. donovani* exposed to DB766 revealed effects similar to those observed in the presence of sterol biosynthesis inhibitors, such as the appearance of vesicles in the cytoplasm, flagellar pocket, and flagellum. *L. donovani* axenic amastigotes were also raised that displayed 12-fold resistance to DB766. These DB766 resistant parasites (DB766R) were not cross resistant to pentamidine, but were hypersensitive to the sterol 14α-demethylase (CYP51) inhibitors ketoconazole and posaconazole (2000-fold more sensitive and over 12,000-fold more sensitive than wild type, respectively). The expression of CYP5122A1, a recently identified cytochrome P450 associated with ergosterol metabolism in *Leishmania,* is dramatically reduced in the resistant parasites as assessed by western blotting. Consistent with these observations in DB766R parasites, CYP5122A1 half knockout *L. donovani* promastigotes were significantly more susceptible to ketoconazole and less sensitive to DB766 than their wild type counterparts. Synergistic activity was also observed in both *L. donovani* axenic amastigotes and intracellular forms with DB766-posaconazole combinations. These studies form the basis for mechanistic hypotheses concerning the antileishmanial action of the AIA DB766 and indicate that DB766-azole combinations may hold promise for the treatment of leishmaniasis.

## 301

### DISCOVERY AND STRUCTURE-ACTIVITY RELATIONSHIPS OF 2-CHLORO-N-(1-HYDROXY-1,3-DIHYDROBENZO[C][1,2]OXABOROL-6-YL)-4-(1H-PYRAZOL-1-YL)BENZAMIDE (AN7973) FOR THE TREATMENT OF AFRICAN ANIMAL TRYPANOSOMIASIS

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Animal African Trypanosomiasis (AAT) is a parasitic disease caused by tsetse fly-transmitted trypanosomes that include *Trypanosoma congolense, T. vivax, T. brucei brucei*. AAT results in serious economic losses in livestock because of reduced productivity, variable clinical signs (e.g. anemia, emaciation) and death. Drug resistance has been an increasing problem in chemotherapy and chemoprophylaxis control. Leveraging the experience of the successful development of a clinical drug candidate against Human African Trypanosomiasis (HAT), we have screened the Anacor benzoxaborole compound library against *T. congolense* (IL-3000) in a 3-day *in vitro* cell viability assay, in order to identify novel chemical entities for AAT compared to the current standard drugs, diminazene and isometamidium. A number of benzoxaborolyl-benzamide analogs were found to show $IC_{50}$ values of less than 100 nM including AN7973, which showed an $IC_{50}$ value of 57 nM. Compounds bearing hydrophobic groups, such as chloro and trifluoromethyl, tended to show potent activity. In contrast, polar substituents, such as carboxy, were not tolerated. Since many compounds showed almost equally potent activity *in vitro*, compounds to be tested *in vivo* were chosen based on the efficacy data against *T. b. brucei* in mice that had been obtained through the HAT study. The selected compounds were tested for their efficacy in an *in vivo* mouse model against *T. congolense* (STIB736/IL1180). Several compounds showed 100% cure lasting > 60 days by intraperitoneal administration at 10 mg/kg for 4 days. AN7973 showed 100% cure at a lower dose of 3 mg/kg for 4 days as well. AN7973 showed potent activity against *T. congolense* both *in vitro* and *in vivo* and appears to be a promising lead for an anti-AAT agent.

## 302

### *LEISHMANIA BRAZILIENSIS* RECOMBINANT HISTONE H2B FOR THE SERODIAGNOSIS OF TEGUMENTARY LEISHMANIASIS

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American tegumentary leishmaniasis is an important endemic disease in Peru and other countries in the New World. It presents a spectrum of clinical manifestations, the principal forms being cutaneous (CL) and mucocutaneous leishmaniasis (MCL). At present, there is not an ideal diagnosis method that matches quality (sensitivity and specificity) and affordable cost for field use. Parasitological diagnosis by microscopy and culture has low sensitivity while DNA-based diagnosis (PCR) is highly sensitive but expensive and technically cumbersome. Hence, a method relying on serological diagnosis is desirable. While serological assays have proved useful for diagnosing visceral leishmaniasis cases which have a marked humoral immune response, there is not such a standardized method available for diagnosing CL and MCL cases. Typically total crude leishmanial antigen is used with variable sensitivities and with cases of cross-reaction. A good candidate for serological diagnosis reported from the Old World is the histone H2B. We hypothesize that serological detection of a recombinant H2B from *Leishmania braziliensis*, the most prevalent species in Peru, would simplify diagnosis, reduce cross-reaction and ultimately allow broad standardization of the method for its use in endemic regions. To this end, the L. *braziliensis* h2b gene was cloned in pET-21a(+) vector and expressed in *E. coli* BL21(DE3) by induction with 1mM IPTG during five hours at 37°C with shaking. Bacteria were lysed and the recombinant protein was purified by nickel affinity chromatography. Serum samples from patients originating from an endemic region were pre-absorbed with *E. coli* lysate and assayed for reactivity by western blot. We have assayed two CL and three MCL patients and found reactivity for the five samples, with apparently higher titers in the MCL form. We did not find cross-reaction with serum from an acute *Trypanosoma cruzi*-infected patient and with a non-endemic negative control donor. Currently, we are analyzing a bigger set of samples, the results of which will be shown at the meeting. In the future we expect that a serological assay based on this protein can be used in the field for epidemiological surveillance of tegumentary leishmaniasis.

## 303

### LIPOSOMAL RESIQUIMOD FOR THE TREATMENT OF *LEISHMANIA DONOVANI* INFECTION

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*Leishmania* is a zoonotic parasite that causes Leishmaniasis; a disease that affects approximately 12 million people worldwide. Leishmaniasis occurs either in its cutaneous (CL) or visceral (VL) form. VL is responsible for the deaths of roughly 50,000 people per year. Treatments exist to treat VL, including intravenous (i.v) delivery of antimony or liposomal amphotericin B, and recently developed oral formulations, such as Miltefosine. While these treatments have been effective, resistant strains can be a problem and require constant development of novel treatment formulations, including those that could potentially eliminate infections through immune-mediated mechanisms. Imidazoquinolines, a family of immunomodulatory toll-like receptor 7/8 agonist compounds, have been quite successful at treating CL, however they have not been utilized for parenteral delivery due to their hydrophobicity. One way to deliver a hydrophobic compound systemically is by incorporation into a drug delivery vehicle, such as a liposome. Using a lipid film hydration method, followed by extrusion, we were able to encapsulate the imidazoquinoline resiquimod and deliver the compound by i.v injection to *L. donovani* infected BALB/c mice. Liposomal resiquimod significantly decreased parasite load in the liver, spleen, and bone marrow while simultaneously stimulating interferon-γ and interlukin-10 production. Histological and *in vitro* analysis showed liposomal resiquimod was non-toxic. Liposomal delivery of FDA-approved resiquimod provides a promising avenue for immune mediated clearance of VL infections.

## 304

### CHARACTERIZATION OF DEVELOPMENTAL STAGE AND STRAIN SPECIFIC SALIVARY ANTIGENS OF *TRIATOMA INFESTANS* AS POTENTIAL EXPOSURE MARKERS

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Antibody responses of animals to saliva of *Triatoma infestans* can be useful indicators of the spatial distribution of triatomines and therefore they could support vector control efforts in endemic Chagas disease areas. As previously presented, salivary protein profiles of *T. infestans* differ among development stages and strains, and due to these differences host antibody responses can also vary. These differences should be taken into account when screening for a potential marker of triatomine exposure. Therefore, our study focused on the antibody response of guinea pigs experimentally exposed to saliva of different development stages of *T. infestans* from Argentina, Bolivia, Chile and Peru. Using IgG antibodies, 1D- and 2D-Western blotting analyses of guinea pig sera revealed a variety of detected salivary antigens from different *T. infestans* populations. IgG antibody responses demonstrated to remain detectable up to several months in animals exposed to triatomines. Thus, antigens recognized by IgG antibodies are not suitable for the detection of bugs at an early stage after insecticide sprayings of vector control programmes. Hence, IgM antibody responses of experimental guinea pigs were analyzed by 1D- and 2D- Western blotting. Despite the variability of IgM recognized antigens a potential exposure marker of 35 kDa was identified using both IgG and IgM antibodies of sera from laboratory experiments and from guinea pigs living in endemic Chagas disease areas of Bolivia. The specificity of this antigen was verified in cross-reactivity experiments, namely if antibodies elicited in guinea pigs by salivary proteins from different hematophagous arthropods cross-reacted in immunoassays with the candidate exposure marker. Sera of guinea pigs exposed to other triatomine species were also used to evaluate if the exposure marker is *T. infestans* specific. Additionally, peptides of a pallidipin-like protein identified as potential exposure marker from our previous study were synthesized and analyzed for their reactivity with guinea pig sera in immunoassays.

## 307

### GENETIC ORIGIN OF HISTIDINE-RICH PROTEIN 2 GENE DELETION IN *PLASMODIUM FALCIPARUM* PARASITES FROM PERU

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A majority of commercially available malaria rapid diagnostic tests (RDTs) detect *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2), which is encoded by *pfhrp2*, a gene located subtelomerically on chromosome 8. Recently, it was determined that approximately 40% of *P. falciparum* isolates from the Peruvian Amazon lacked *pfhrp2*, leading to false-negative RDT results. We hypothesized that *pfhrp2*-deleted parasites in Peru may have derived from a single genetic event and expanded. To test this hypothesis, we examined historical samples collected between 1999 and 2005 to evaluate the haplotype structure of *pfhrp2* using microsatellite markers flanking the gene. Our data shows evidence for genetic deletions in at least four distinct *pfhrp2* haplotypes corresponding to four different clonal lineages of parasite populations collected in 1999-2001. Furthermore, there is evidence for recombination of these *pfhrp2* haplotypes in subsequent years (2003-2005) resulting in the emergence of hybrid haplotypes. These findings indicate that the genetic origin of *pfhrp2* deletion was not a rare event, as we had hypothesized, but may have occurred multiple times independently in parasites of different genetic backgrounds, partially explaining the widespread presence of *pfhrp2*-deleted parasites in Peru. Future investigations are necessary to determine the selective force(s), if any, acting on these population(s) that favor expansion of *pfhrp2* negative parasites.

## 308

### QUANTITATIVE DETECTION OF *PLASMODIUM FALCIPARUM* HISTIDINE RICH PROTEIN 2 IN SALIVA OF CHILDREN WITH MALARIA

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Malaria is a global health priority with a heavy burden of fatality and morbidity. Improvements in field diagnostics are needed to support the agenda for malaria elimination. Saliva has shown significant potential for use in non-invasive diagnostics, but the development of off-the-shelf saliva diagnostic kits requires best practices for sample preparation and quantitative insight on the availability of biomarkers and the dynamics of immunoassay in saliva. This study measured the levels of the PfHRP2 in patient saliva. Matched samples of blood and saliva were collected between March and August, 2011 from forty patients at the ER and OPD of the pediatric unit of Korle Bu Teaching Hospital. Parasite density was determined from thick-film blood smears. Concentrations of PfHRP2 in saliva of malaria-positive patients were measured using a custom chemiluminescent ELISA in microtitre plates. Forty negative-control patients were enrolled. Saliva samples were stabilized with protease inhibitor.Of the forty patients with microscopically confirmed *Plasmodium falciparum* malaria, thirty seven tested positive for PfHRP2 in the blood using rapid diagnostic test kits, and forty for PfHRP2 in saliva. All negative-control samples tested negative for salivary Pf HRP2. The ELISA greed with microscopy with 100 % sensitivity and 100 % specificity. Salivary levels of PfHRP2 ranged from 15 to 1,162 pg/mL in the malaria-positive group. Saliva is a promising diagnostic fluid for malaria when protein degradation and matrix effects are mitigated. Systematic quantitation of other malaria biomarkers in saliva would identify those with the best clinical relevance and suitability for off-the-shelf diagnostic kits.

## 309

### ANALYSIS OF THE PFHRP2 GENETIC DIVERSITY IN THREE AFRICAN COUNTRIES AND IMPLICATIONS FOR RAPID DIAGNOSTIC TEST EFFICACY

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The Senegalese National Malaria Control Program has recommended the use of Rapid Diagnostic Tests (RDTs) that target the Histidine Rich Protein 2 (HRP2), specific to *Plasmodium falciparum*, to diagnose malaria cases. The target antigen has been shown to be polymorphic which may explain the variability in HRP2-based RDT results reported in field studies. The genetic diversity of the pfhrp2 gene has not been investigated in depth in many African countries, including Senegal. The goal of this study is to determine the extent of polymorphism in pfhrp2 in three unique African populations: Senegal, Mali, and Uganda, and discuss the implications of these findings on the utility of RDTs based upon HRP2 detection. Sequencing data from the pfhrp2 locus was used to analyze the genetic diversity of this gene among three African populations Senegal, Mali, and Uganda with divergent transmission dynamics and malaria parasite ecology. Both single nucleotide polymorphisms (SNPs) in the pfhrp2 gene and amino acid repeat polymorphism were characterized, and parameters of genetic diversity in these populations were assessed. We observed extensive repeat length polymorphism in PfHRP2 antigen, and 67 mutations in the pfhrp2 gene including: Synonymous and Non-Synonymous single nucleotide polymorphisms (SNPs), insertions and deletions (INDELS), frame shifts, were identified; however, little diversity was observed at the nucleotide level ($\pi$=0.0000, $\pi$=0.00233, $\pi$=0.00289 for Senegal, Mali and Uganda respectively). Similar patterns were observed in the number, organization and the type of predicted amino acid repeats in the protein among the three populations, characterized by an occurrence of the Type 2, Type 4 and Type 7 repeats in the all populations studied. Type 4(AHH) and Type 7 (AHHAAD) are significantly different between the 3 populations with Pvalue=0.0067 and P=0.0287 respectively. The frequency and distribution of amino-acids repeats shows inter and intra-geographic variation in the PfHRP2 protein. The results provide insight about the genetic diversity of the pfhrp2 gene; and, based on the low genetic diversity observed, pfhrp2 seems to be a good candidate for RDTs design. However, since deletions of this gene has been observed in some endemic countries, it is crucial to focus on other essential genes as a targets for diagnostic tools.

## 310

### DETECTION OF *PLASMODIUM* SPECIES CAUSING MALARIA IN LILONGWE, MALAWI

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Malaria RDT have played a major role in malaria management; particularly in providing blood based diagnosis in remote locations where microscopy is unavailable. These tests are fast and easy to perform and do not require electricity or specific equipment. As part of strengthening malaria diagnostics in Malawi, the Ministry of Health recommends use of RDTs in health care delivery system. However, microscopy remains a gold standard test for malaria. All patients with suspected uncomplicated malaria should have a confirmed diagnosis with RDT before antimalarial treatment is administered. Based on field performance evaluations that assessed performance, quality control and production capacities of the manufacturing companies of malaria RDTs, the Ministry of Health recommended two brands of Histidine Rich Protein 2 (HRP-2), RDTs for use in Malawi. These are SD Bioline malaria Ag *Pf* and the New Paracheck malaria Ag *Pf.* All these RDTs are able to detect only *Plasmodium falciparum.* However, other species have been reported to exist in the

country and there is need to find proper RDTs that will be able to detect and other species including *P. falciparum*. This study recruited a total of 250 adult and infants in Lilongwe, Malawi. The samples were processed at Bwaila Hospital Laboratory, UNC Project Laboratory and the University of North Carolina in Chapel Hill. Study results showed that the overall sensitivity and specificity of the Paramax-3 RDT used in the study were 100% and 83% respectively. However, it was observed that the RDT test was not able to identify the *P. ovale*, and in some cases, the RDT test was positive for *P. falciparum* when the PCR identified the species as *P. ovale*. No *P. vivax* was detected both by RDT and PCR. This study was able to detect and identify the presence of *P. malariae* and *P. ovale* in Malawi apart from the *P. falciparum*. There were no significant differences in between microscopy results compared to both the RDT and the PCR, with 94% and 98% sensitivities of R1 and R2 compared to RDT, as well as 94% and 96% sensitivities for R1 and R2 compared to PCR respectively. Both R1 and R2 had low specificities for example, R1 had 72% and R2 had 80% compared to RDT. Comparing R1 and R2 to PCR, the sensitivities were 64.9% and 67.2% respectively. However, the readers had difficulties differentiating the different species microscopically. The history of antimalarial treatment had no significant effect in the outcome of the results in both the RDT and PCR.

## 311

### UTILIZATION OF MALARIA DIAGNOSTIC TESTS AND RECEIPT OF ANTIMALARIAL DRUGS BY FEBRILE PATIENTS ATTENDING OUTPATIENT DEPARTMENTS OF HEALTH CENTER IVS IN MUKONO DISTRICT, UGANDA

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Malaria remains a major public health problem in Uganda with annual estimates of 10 million cases and 43,000 deaths. Demonstration of the presence of malaria parasites prior to treatment with anti-malarial drugs is paramount to effective malaria case management. The aim of this study was to describe patients' factors associated with utilization of malaria diagnostic tests among febrile patients suspected of malaria and to help understand the role of utilization of malaria diagnostic tests in the receipt of anti-malarial drugs in a bid to better inform policy-makers on possible measures to minimize anti-malarial drug wastage at public health facilities. In a cross-sectional study design, client-exit interviews with febrile patients were conducted at health center IVs in Mukono district. Data were obtained with the aid of an interviewer-administered predetermined semi-structured questionnaire. Data entry and analysis were done using Epi-Data version 3.2 and STATA version 10.0 respectively. Frequencies and proportions were used to describe the sample population; chi square was used in two by two tables, odds ratios as the measure of association and an alpha level of 0.05 was used in all significance tests. Out of 472 potential participants screened for eligibility, 408 consented, almost half of whom were female (252, 61.8%) aged less than five years (120, 29.4%). There were no statistically significant differences between utilizers and non-utilizers in most characteristics except age, time of arrival at the health center, history of indoor residual spraying and overall satisfaction with services at out-patient-departments. Out of the 408 respondents, 359(88%) utilized malaria diagnostic tests and 241(59%) received anti-malarial drugs while 12(3%) neither utilized malaria diagnostic tests nor received anti-malarial drugs. In the adjusted analysis, utilizers were 75% less likely to receive anti-malarial drugs than non-utilizers after controlling for age, sex and type of place of residence (OR: 0.25, 95%CI: 0.09 , 0.66). Utilization of malaria diagnostic tests indeed has a bearing on the receipt of anti-malarial drugs. Efforts to minimize anti-malarial drug wastage should be therefore geared towards increasing utilization of malaria diagnostic tests.

## 312

### IMPROVING DIAGNOSTIC CAPACITY FOR MALARIA AND MANAGEMENT OF FEBRILE ILLNESS IN UGANDA BY TRAINING LABORATORY HEALTH CARE WORKERS IN MALARIA DIAGNOSIS BY MICROSCOPY

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Malaria diagnosis with either microscopy or a rapid diagnostic test (RDT) is recommended for all cases of fever in Uganda to improve patient care, reduce unnecessary antimalarial use, improve surveillance, provide confirmation of treatment failures and allow for other diagnosis to be sought in negative cases. Coverage of malaria diagnostic services remains very low in Uganda and quality microscopy services are severely lacking. Since 2009, Uganda Malaria Surveillance Project (UMSP) in partnership with the National Malaria Control Program have trained laboratory personnel in malaria diagnosis. Training targeted all cadres of laboratory personnel from all health facilities in Uganda. The objective was to improve diagnosis capacity for malaria and management of febrile illness by training laboratory health care workers in malaria diagnosis by microscopy. The methods of Training consisted of a three-day course conducted at a centrally located district hospital or Health Centre IV with 75% of the time dedicated to practical sessions. Training impact was measured through a written examination and evaluation of the quality of blood slide preparation and accuracy of field microscopy. A total of 1462 laboratory personnel were trained from 21 districts. Average test scores improved from 41% to 75% ($p<0.001$). Sensitivity improved from 84% to 95% ($p<0.001$) and specificity improved from 87% to 97% ($p<0.001$). The proportion of well prepared blood smears improved from 6% to 75% ($p<0.001$). Therefore the refresher training significantly improved malaria diagnosis accuracy.

## 313

### PRESENTING ATYPICAL LYMPHOCYTES AND *THROMBOCYTOPENIA* IN MALARIA INFECTION RESEMBLE TO DENGUE INFECTION

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Uncomplicated *Plasmodium falciparum* and *P. vivax* malaria patients usually present with acute febrile illness, atypical lymphocytosis and thrombocytopenia similar to dengue infection. To investigate the similarity manifestation, we retrospectively studied atypical lymphocytes (AL), atypical lymphocytosis (ALO) and platelet count in 1,310 uncomplicated malaria patients who admitted in the Bangkok Hospital for Tropical Diseases. 718 *P. falciparum* and 592 *P. vivax* malaria patients were enrolled into the study. In *P. falciparum* malaria patients, upon admission, AL and ALO were found in 53.2% and 5.7% of the patient, respectively (range 0-10%). While in *P. vivax* malaria patients, AL and ALO were seen in 55.4% and 9.5% of the patients, respectively (range 0-14%). After receiving antimalarial therapy, AL and ALO declined in both groups. However, AL and ALO remained occurred until the 4$^{th}$ week after admission. Interestingly, we also found that all patients presented with thrombocytopenia on admission. Although initial platelet counts were significantly lower than normal in both study groups, they slowly increased significantly over time and approached normal levels by days 28 post-treatment. In conclusion, AL or ALO as well as low platelet counts may be found in uncomplicated *falciparum* and *vivax* malaria mimicking dengue infection. In tropical countries where both dengue and malaria are endemic, presence of AL or ALO in any acute febrile patients with thrombocytopenia (similar to the findings in dengue) malaria could not be excluded. Particularly if the patients have risk of malaria infection, confirmative microscopic examination for malaria should be carried out.

## 314

### ASSOCIATED FACTORS OF CIRCULATORY SHOCK IN ADULT PATIENTS WITH SEVERE *FALCIPARUM* MALARIA

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Infection with *Plasmodium falciparum* is a life-threatening medical condition particularly in severe malaria patients. Shock is a complication that frequently occurs and needs immediately clinical management. To investigate the risk factors for the development of shock in adult patients with severe *falciparum* malaria. We conducted a retrospective unmatched case-control study of the adult patients admitted to the Bangkok Hospital for Tropical Diseases, Thailand, between the years 2000-2010. One hundred patients with severe *falciparum* malaria and shock and 100 patients with severe malaria without shock were studied. Demographics, presenting signs and symptoms and initial laboratory data of those patients were analyzed. By statistical method, we found that 5 risk factors were identified as indicators of shock. These included female gender (OR 6.16, 95% C.I. 3.17 - 11.97), red cell distribution width (RDW) >15% (adjusted OR 2.90, 95% C.I. 1.11 - 7.57), anorexia (adjusted OR 2.76, 95% C.I. 1.03 - 7.39), hypoalbuminemia (adjusted OR 2.19, 95% C.I. 1.10 - 4.34), and BUN-Creatinine ratio >20 (adjusted OR 2.38, 95% C.I. 1.22 - 4.64). It is interesting that diarrhoea was found to be a protective factor (adjusted OR 0.33, 95% C.I. 0.14 - 0.78). We concluded that female gender, RDW >15%, anorexia, hypoalbuminemia, and BUN-Creatinine ratio >20 were risk factors of shock development in severe *P. falciparum* malaria patients. Further studies need to be compared with this data from different geographical areas, to construct practical measures to address potentially shock indicators in different settings.

## 315

### PRESENTING *SCHIZONTEMIA* AND SEVERITY OUTCOME IN ADULT PATIENTS INFECTED WITH *PLASMODIUM FALCIPARUM* MALARIA

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Malaria, an infectious disease caused by protozoans of the genus *Plasmodium* continues to be a major health problem, particularly in the tropics and sub-tropics. Hyper*parasitemia* is a laboratory feature that commonly occurs in severe malaria patients. However, some malaria patients may undergo to severe malaria with initial low *parasitemia*. Schizontemia is a factor that may be caused of suffering in malaria patients. To investigate whether schizontemia associated with severe malaria condition and could be used as an indicator for predicting severe malaria. We retrospectively studied initial clinical and laboratory presentations upon admission in malaria patients admitted in the Bangkok Hospital for Tropical Disease. According to WHO criteria, 250 uncomplicated *falciparum* malaria cases and 250 severe *falciparum* malaria cases were enrolled in to the study. Based on statistical analysis method we found that presenting schizontemia were only detected in 99 severe malaria patients (39.6%). Moreover, presenting schizontemia also showed correlation with initial asexual parasite density. We concluded that presenting schizontemia in *falciparum* malaria might be helpful for clinicians in order to treat malaria patients since schizont stage was not found in uncomplicated *falciparum* malaria in this study. Further studies need to be carried out to compare with this data from different geographical settings, to construct a practical measure to address potentially presenting schizontemia in severe malaria patients in different settings.

## 316

### MULTIPLEX QPCR FOR DETECTION AND ABSOLUTE QUANTIFICATION OF MALARIA

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We describe development of an absolute multiplex quantitative real-time PCR for detection of *Plasmodium spp.*, *P. falciparum* and *P. vivax* targets. Important qPCR experimental details and information that are important for the performance and reliability of PCR assay were investigated. Inhibition studies were performed to test and compare co-purification of PCR inhibitors in samples extracted from whole blood using either manual or automated methods. To establish the most optimal qPCR reaction volume, volume titration of the reaction master mix was performed starting at 10µL to 1µL of the reaction master mix with 1µL of template DNA in each assay reaction. As the reaction volume decreased, qPCR assays became more efficient with 1µL reaction master mix being the most efficient. For more accurate quantification of parasites in a sample, we developed plasmid DNAs for all the three assay targets for absolute quantification. All of absolute qPCR assays performed with efficiency of more than 94%, $R^2$ values greater than 0.99 and the STDEV of each replicate was <0.167. Linear regression plots generated from absolute qPCR assays were used to estimate the corresponding parasite density from relative qPCR in terms of parasite/µL. One copy of plasmid DNA was established to be equivalent to 0.1 parasite/µL for *Plasmodium spp.* assay, 0.281 parasites for *P. falciparum* assay and 0.127 parasite/µL for *P. vivax* assay. This study demonstrates for the first time use of plasmid DNA in absolute quantification of malaria parasite. The use of plasmid DNA standard in quantification of malaria parasite will be critical as efforts are underway to harmonize molecular assays used in diagnosis of malaria.

## 317

### USE OF PET-PCR FOR THE MOLECULAR DETECTION OF MALARIA PARASITES IN HAITI NATIONAL MALARIA SURVEILLANCE STUDY

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We recently described a real-time PCR assay known as photo-induced electron transfer (PET)-PCR which relies on self-quenching primers for the detection of *Plasmodium spp.* and *P. falciparum*. PET-PCR assay is robust, less expensive and easier to use in resource limited countries when compared to currently available real-time PCR methods. Here, we investigated the potential of PET-PCR for molecular detection of malaria parasites in 2989 dried blood spots collected for national malaria Tracking Results Continuously (TRaC) community survey in Haiti, conducted in 2011. DNA from the dried blood spots was extracted using the QIAGEN method. All the 2989 samples were screened using the PET-PCR assay in duplicates. Samples with a cycle threshold (CT) of 40 or less were scored as positive. A randomly selected subset of the total samples (546) was tested using a nested PCR assay for confirmation. In addition, these same samples were also tested using a TaqMan-based PCR assay. A total of 12 out of the 2989 samples screened (0.4%) were found to be positive by PET-PCR. These same samples were also found to be positive by the nested and TaqMan-based methods. The nested PCR detected an additional positive sample that was not detected by either PET-PCR or TaqMan-based PCR method. The sensitivity and specificity of the PET-PCR compared to the nested PCR, as a gold standard, were found to be 92.3% (95% CI:

69.9%-100%) and 100% (95% CI: 99.1%-100%), respectively. Similar sensitivities and specificities were obtained for the TaqMan-based real-time PCR. PET-PCR yielded comparable results with other sensitive PCR methods and can be considered for rapid screening of large scale samples in surveillance studies.

## 318

### DETECTION OF ANTIMALARIAL DRUG RESISTANCE: PERFORMANCE OF THE NOVEL HEMOZOIN SENSITIVITY ASSAY IN GABON

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Antimalarial-drug resistance is a threat for malaria control. Resistance has been described for many drugs, including the first-line treatment with artemisinins. *In vitro* antimalarial sensitivity testing is crucial to detect and monitor drug resistance. Currently available sensitivity assays have some drawbacks, including the detection of resistance in field isolates (e.g.: none detects artemisinin resistance). The recently developed hemozoin (Hz) detection assay showed to be able to overcome some of the limitations of existing assays. Promising results were obtained using *P. falciparum* continuous cultures in the laboratory. In this study we have determine the utility and performance of the Hz detection by directly culturing *ex vivo* blood samples from malaria Gabonese patients. Infected red blood cells were isolated and immediately incubated for 72-hours with increasing concentrations of chloroquine, artesunate and artemisinin. On site, a flow cytometer – Cyflow (Partec, Munster) was easily modified to detect Hz light depolarization. The percentage of Hz-containing infected red blood cells determined by flow cytometry was used as maturation indicator. Measurements were done at 24, 48 and 72 hours of incubation. Preliminary results showed that by assessing the percentage of Hz-containing infected red blood cells parasite maturation and antimalarial-drug inhibitory effects could already be detected after 24-hours of incubation. Inhibitory concentrations of 50% (IC50) were calculated at the same time-point. In the case of artemisinin compounds $IC_{50}$ values were higher than the ones previously described using the Histidine-rich protein 2 (HRP2) assay. This might be a consequence of the assay's conditions, since it has been described that some drugs show an increased inhibitory concentration when greater numbers of parasites are inoculated. In the HRP2 assay parasite densities are adjusted to 2500 parasites/µl, while in the Hz detection assay they were not adjusted, thus analyzed samples had parasite densities ranging from 2400 parasites/µl up to 79200 parasites/µl. These results suggest that the rapid (24-hour), novel Hz assay could be used to determine the sensitivity/resistance pattern of the parasites in clinical isolates. This would be an useful tool not only in malaria endemic countries but also for rapid resistance testing in returning travellers.

## 319

### PREVALENCE OF DRUG RESISTANCE ASSOCIATED MUTATIONS IN CLINICAL ISOLATES OF *PLASMODIUM VIVAX* FROM SOUTHERN PAKISTAN

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Antifolate antimalarial drugs sulphadoxine-pyrimethamine (SP) are the mainstay of malaria control in Pakistan. Though, not recommended, it is still being used largely in the public sector against *Plasmodium vivax*.Extensive use of SP indicates possibility of accumulation of drug resistance associated mutations in SP binding sites of *P. vivax*, encoded by *dihydrofolate reductase (dhfr)* and *dihydroptereoate synthethase* (*dhps*) genes. The aim of this study was to identify baseline frequencies of single nucleotide polymorphisms (SNPs) and prevalence of SP resistance associated mutations in clinical isolates of *P. vivax* (n=131) from Karachi, Sindh and Balochistan province. Nested PCR followed by direct sequencing and comparison with wild type reference sequences was performed. In *dhfr*, mutations were observed at codons F57L, S58R and S117N/T while novel non-synonymous mutations were observed at codon positions N50I, G114R and E119K .Two mutations, N50I and 117T were observed for the first time in Pakistan. The 50I mutation signifies intra species recombination of *P. falciparum* and *P. vivax* while 117T, isolated globally from treatment failure cases, shows the extent of SP pressure on *P. vivax*. In *dhps*, mutations were observed at codon position A383G and A553G while non-synonymous mutations were observed at codon positions S373T, E380K, P384L, N389T, V392D, T393P, D459A, M601I, A651D and A661V. Results from this study provide evidence that increasing number of SP resistance associated mutant alleles, comparable to those reported worldwide, are circulating in southern Pakistan. These alleles may play a significant role in transmission of resistant strains via human parasite reservoirs exacerbating extensive drug pressure on *P.vivax* and possibly making SP defunct for future use.

## 320

### *PLASMODIUM FALCIPARUM* FIELD ISOLATES EX VIVO ANTIMALARIAL DRUG RESPONSE IN THIES (SENEGAL)

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*Plasmodium falciparum* malaria continues to be a major global cause of mortality and morbidity. Malaria treatment and control has been complicated by the emergence of resistance to widespread antimalarial drug use. Simple assays to monitor parasite drug response in clinical samples are important, as they can detect drug resistance before it becomes clinically apparent as well as inform changes in treatment policy to help prevent the spread of resistant parasites. We surveyed malaria cases in a clinic in Thies, Senegal from 2008-2012 using DAPI-based ex vivo drug assay to test *P. falciparum* response to amodiaquine, chloroquine, quinine, pyrimethamine, lumefantrine, piperaquine, mefloquine and artemisinin derivates in approximately 500 clinical isolates. Mutations in pfcrt and pfmdr1 were associated with changes in drug response, and we observed strong concordance between the ex vivo and *in vitro* $IC_{50}$s of culture adapted parasites. Thus surveillance of ex vivo drug sensitivity assays should be an integral part of the planned malaria control program so that resistance dynamics can be assessed and the most effective treatment can be selected or modified.

## 321

### FIELD VALIDATION OF CANDIDATE MOLECULAR MARKERS OF ARTEMISININ RESISTANCE IN THAILAND

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The emergence of artemisinin resistance in Southeast Asia threatens the efficacy of artemisinin-based combination therapies (ACTs) and prospects of successful malaria elimination. Molecular markers of artemisinin resistance would provide useful tools for surveillance of resistance and help guide containment and elimination efforts. The first genome-wide association study of artemisinin resistance in clinical *Plasmodium falciparum* infections identified two candidate single nucleotide

polymorphisms (SNPs) on chromosomes 10 and 13 (MAL10-688956 and MAL13-1718319), that were significantly associated with delayed parasite clearance after treatment with oral artesunate. These SNPs were genotyped by pyrosequencing in 400 patient samples from eight sites in Thailand, collected between 2006 and 2011 as part of routine WHO Therapeutic Efficacy Surveys monitoring ACT treatment outcomes. Parasite clearance at these sites ranged from a half-life of 2 hours, corresponding to sensitive parasites, to up to 7 hours in some regions, comparable to slow-clearing artemisinin-resistant parasites described in western Cambodia. Prevalence of both SNPs varied greatly by region, ranging from less than 10% to fixation at 100% across sites. The association between the presence of each SNP on day 0 prior to treatment with the prevalence of *parasitemia* on day 3 and PCR-confirmed recrudescence after treatment was estimated as a means of validating these candidate molecular markers as predictors of clinical artemisinin resistance. Odds ratios for association between SNPs and day 3 *parasitemia* will be reported. This study represents the first attempt to validate these candidate molecular markers in an independent set of clinical samples from areas of Thailand with known artemisinin resistance.

## 322

### ANALYSIS OF THERAPEUTIC EFFICACIES OF AMODIAQUINE-ARSTESUNATE AND ARTEMETHER-LUMEFANTRINE FOR TREATMENT OF UNCOMPLICATED *FALCIPARUM* MALARIA IN BURKINA FASO FIVE YEARS AFTER THEIR IMPLEMENTATION

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Since 2005, Burkina Faso adopted artesunate plus amodiaquine (ASAQ) and artemether-lumefantrine (AL) as first-line treatment for uncomplicated malaria. Despite improvement in that treatment, malaria remains the first cause of morbidity and mortality in the country. This study aimed to analyze the therapeutic efficacies of ASAQ and AL for the treatment of uncomplicated *falciparum* malaria in Burkina Faso five years after their adoption. Per-protocol individual data from four randomized clinical trials supported by IRSS-DRO Bobo Dioulasso in 2006, 2008, 2009 and 2010, including 1076 patients with uncomplicated *Plasmodium falciparum* malaria, treated with the recommended regimen of AL or ASAQ, were analyzed according to WWARN analytical methods. Patients benefited from a clinical and biological 28-day follow-up and performed on days 2, 3, 7, 14 and 28 to evaluate clinical and parasitological outcomes. Treatment failures have been corrected by PCR. Results: Using WWARN analytical methods, the unadjusted Kaplan-Meier survival estimates are 76.4% (95% CI (72.5-79.8)) in the AL group (N=544) and 87.1% (95% CI (83.9-89.7)) in the ASAQ group (N=532). After PCR correction, AL was less efficacious than ASAQ respectively 95.8% (95% CI (93.6-97.3)) vs 98.2% (95% CI (96.6-99.1)); OR=0,486 (95% CI (0.217-1.089). There was no significant correlation between the occurrence of recrudescent at day 28 end-point and study year in two groups (coefficient<0.1). Conclusion: AL and ASAQ remain effective as treatment for uncomplicated malaria according to WHO recommendations, though AL was inferior in preventing recrudescent for 28-day follow-up.

## 323

### ASSESSMENT OF RESISTANCE MARKERS LEVEL FOR ARTESUNATE + AMODIAQUINE COMBINATION FOR THE TREATMENT OF THE UNCOMPLICATED MALARIA IN MAFERINYAH, GUINEA

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The use of the Amodiaquine in monotherapy, is associated with the selection of resistance markers (*pfcrt* K76T and *pfmdr1*86Y). Although there is not documented resistance, no resistance markers to artemisin derivatives it is important to assess the impact of artemisin based combination therapy (ACT) on the selection of markers associated with partner molecules. This study was undertaken **t**o evaluate the efficacy of Artesunate+Amodiaquine combinationin the treatment of uncomplicated malaria in Maferinyah, Guinea Conakry; - To search for punctual mutations on Amodiaquine resistance genes (*Pfcrt* 76T and *Pfmdr1*-86Y). - To assess for polymorphisms (MSP1, MSP2 and CA1) in order to discriminate new infection versus recrudescence. We assessed *invivo* efficacy of Artsunate+Amodiaquine (AS+AQ) on subjects aged from 3 months to 45 years living in Mafrinyah, near Conakry in Guinea. The efficacy of AS+AQ has been evaluatedby WHO 28 days standard *invivo* test. Polymorphisms (MSP1, MSP2 and CA1) and punctual mutations on Amodiaquine resistance genes (*Pfcrt*76T and *Pfmdr1*-86Y) have been determined by PCR. A total of 93 samples have been randomly selected before treatment and 11 samples with *parasitemia* after treatment have been analyzed. Baseline frequencies of *Pfcrt*76T and *Pfmdr1* mutations were respectively 67.7% (63/93) and 31.1% (28/93). These frequencies after treatment were respectively 50% for *Pfcrt*76T and 54% for *Pfmdr1* 86Y. In conclusion, these data show an increased baseline level of *Pfcrt*76T gene and a significant selection of AQ molecular maker trough AS+AQ.

## 324

### *IN VIVO* EFFICACY AND MOLECULAR RESISTANCE MARKERS OF SULFADOXINE-PYRIMETHAMINE FOR INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY — MANSA, ZAMBIA, 2010

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Intermittent preventive treatment of malaria in pregnancy (IPTp) with sulphadoxine-pyrimethamine (SP) reduces adverse effects of malaria infection. Emergence of SP-resistant *Plasmodium falciparum* threatens this strategy. The quintuple mutant haplotype, mutations in the *dhfr* and *dhps* genes, is associated with SP treatment failure in non-pregnant patients. Zambia has implemented its SP IPTp program since 2003. We sought to determine the efficacy of SP IPTp and the presence of the quintuple mutant, neither previously described among HIV-negative pregnant women in Zambia. In Mansa, Zambia, HIV-negative pregnant women presenting to antenatal clinic for the 1st dose of SP IPTp with asymptomatic *parasitemia* were enrolled and tested for *parasitemia* weekly for 7 weeks. Outcomes were parasitological failure (PF, *parasitemia* during follow-up), and adequate parasitological response (APR, no *parasitemia* during follow-

up). Polymerase chain reaction (PCR) distinguished recrudescence (failure) from reinfection, and identified molecular markers of SP resistance. Survival analysis was done; those who had incomplete follow-up (at least one follow-up day) or reinfection were censored. Of the 108 women enrolled, 51 (46%) completed the study, 34 (31%) had incomplete follow-up, 7 (6%) reinfection, and 17 (16%) were lost to follow-up after day 0 (LTFU). Of those who completed the study, PF occurred in 8 (16%), and APR in 43 (84%). For the 92 women included in survival analysis, median age was 20 years (range 15-39), median gestational age was 22 weeks (range16-28), and 57% were primigravid. There was no difference in time to failure in primigravid versus multigravid women. Of the 84 women with complete haplotype (includes those LTFU), 53 (63%) had quintuple or sextuple mutants. PF occurred in 22% of women with quintuple mutation versus 0% without quintuple mutation (p=0.44). While underpowered and possible bias due to high LTFU, this study shows low failure rates and incomplete penetration of the quintuple mutant. The threat of SP resistance looms, but SP IPTp may remain efficacious in Mansa.

## 325

### MOLECULAR-BASED ANTIMALARIAL RESISTANCE MONITORING FOR *PLASMODIUM FALCIPARUM* IN NICARAGUA

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*Plasmodium falciparum* malaria is a potentially fatal disease; the regular monitoring of antimalarial efficacy is essential to inform national malaria policies. In Nicaragua, chloroquine (CQ) and sulphadoxine-pyrimethamine (SP) are used as primary and secondary drugs, respectively, for treating uncomplicated *P. falciparum* malaria. Since *P. falciparum* incidence in Nicaragua is extremely low (236 cases in 2012), it is extremely difficult to conduct WHO-recommended *in vivo* efficacy trials to assess the efficacy of CQ and SP. In order to monitor antimalarial resistance, the Ministry of Health and its partners implemented a molecular-based antimalarial resistance surveillance system in 2010. Six sentinel sites in the Región Autónoma Atlántica Norte, where most *P. falciparum* malaria cases occur, were established in mid 2010. Following national guidelines, all *P. falciparum* malaria cases are admitted as inpatients for laboratory confirmation and treatment. A sample in filter paper is collected upon admission from all patients and, together with the malaria smear, is later sent to the national reference laboratory, Centro Nacional de Diagnostico y Referencia (CNDR), in Managua. CNDR staff confirms *P. falciparum* infection using microscopy and processes filter papers to evaluate for mutations commonly associated with CQ (*pfcrt*) and SP (*dhfr* and *dhps*) resistance by DNA sequencing. A total of 84 samples (28 in 2010 and 56 in 2011) were collected and sequenced for molecular markers. No resistance alleles known to be associated with CQ or SP resistance were detected. This finding also highlights how the use of molecular-based antimalarial resistance surveillance for both CQ and SP can provide valuable information to monitor for antimalarial resistance in countries such as Nicaragua, where malaria incidence has decreased to levels that impair the ability to conduct *in vivo* trials due to low number of patients eligible for enrollment.

## 326

### PFDHFR AND PFDHPS GENE MUTATIONS ASSOCIATED TO CHEMORESISTANCE IN *PLASMODIUM FALCIPARUM* ISOLATES FROM LUBANGO, ANGOLA

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Malaria is a major parasitic disease. *Plasmodium falciparum* parasites are responsible for severe morbidity malaria cases and its chemoresistance is notorious. Thus, it becomes necessary to know the sensitivity of *P. falciparum* parasites to sulphadoxine / pyrimethanine (SP), to evaluate the effectiveness of pregnant women intermittent preventive treatment (IPT) used in Angola since 2006 as well as the appropriateness of IPT introduction in children under 5 years old, in this country. For this purpose, among the 107 blood samples collected from malaria patients in Lubango, Angola, and subjected to DNA sequencing we observed: 46% with double mutant haplotypes ACNRNVI in codons 59 and 108; 27% with double mutations in codons 51 and 108 showing the haplotype ACICNVI, 17% triple mutant at codons 51, 59 and 108 with haplotypes ACIRNVI; 2% triple mutant at codons 50, 51, 108 and 59, 108, and 164 with haplotypes ARICNVI ACNRNVL; 6% with a single mutation at codon 108, showing ACNCNVI haplotype and; only one wild isolate - ACNCSVI (2%). The 108N mutation (change of serine for asparagine - S108N) in *pfdhfr* gene - a mutation described as the predecessor for all resistant emergence strains - was the most prevalent (98%) followed by 59R (65%) and 51I (47% ) mutations. Regarding *pfdhps* gene, a total prevalence of 437G mutation, with the exception of one sample (4%) who presented wild haplotype SAKAA were noted. Among the mutant samples, 88% had a single mutation presenting haplotype SGKAA; 6% showed double mutants at codons 437 and 540 originating haplotype SGEAA and; 6% displayed also a double mutant but at codons 436 and 437, with haplotype AGKAA. Quadruple (triple *pfdhfr* + single *pfdhps*) and triple mutants (double *pfdhfr* + single *pfdhps*) were recorded. Considering the significant percentage of *P. falciparum* parasites circulating in Lubango with a resistance profile associated with pyrimethamine and a tendency to sulphadoxine tolerance, we conclude that the IPT should not be effective in endemic areas of Angola.

## 327

### DECREASED EX VIVO SENSITIVITY TO ARTEMISININ AND AMODIAQUINE AMONG *PLASMODIUM FALCIPARUM* PARASITES IN THIÉS, SENEGAL

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*Plasmodium falciparum* malaria continues to be a major global cause of mortality and morbidity. Malaria treatment and control has been complicated by the emergence of resistance to widespread antimalarial drug use. Simple assays to monitor parasite drug response in clinical samples are important, as they can detect drug resistance before it becomes clinically apparent as well as inform changes in treatment policy to help prevent the spread of resistant parasites. We tested parasite drug responses and genotyped drug resistance-associated mutations in approximately 400 *P. falciparum* malaria infections in Thiés, Senegal between 2008 and 2011. Over this time period, parasites became increasingly more resistant to both amodaquine and artemisinin, two

compounds deployed in artemisinin combination therapies (ACTs) in Senegal beginning in 2006. Additionally, the prevalence of several known resistance-associated mutations in *pfcrt* and *pfmdr1* also increased between 2008 and 2011. Increased amodiaquine resistance was associated with sustained, highly prevalent mutations in *pfcrt*, and one mutation in *pfmdr1* - Y184F - was associated with lowered parasite response to artemisinin. These data support the hypothesis that the use of amodiaquine and artemisinin derivatives in combination therapies is selecting for increased drug tolerance in this population. Thus surveillance using *ex vivo* drug sensitivity assays should be an integral part of the planned malaria control program, so that resistance dynamics can be assessed and the most effective treatment can be selected or modified.

## 328

### A REPLICATION GENOME-WIDE ASSOCIATION STUDY OF THE GENETIC BASIS OF DELAYED PARASITE CLEARANCE FOLLOWING TREATMENT WITH ARTEMISININS

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Ongoing efforts to contain artemisinin-resistant malaria are hampered by the lack of tools to gauge the extent and direction of its spread. A molecular assay to detect markers of artemisinin resistance would be a highly valuable surveillance tool, but the genetic basis of resistance is unknown. An initial genome-wide screen with a relatively sparse set of markers identified single nucleotide polymorphisms (SNPs) on multiple chromosomes that were associated with delayed parasite clearance, as well as several regions of the parasite genome under recent positive selection. However, replication studies and denser coverage of the genome will be required to more finely map the location of genes involved in artemisinin resistance. In this study, we will replicate the genome-wide association study in an independent set of samples collected during artesunate efficacy studies conducted in Cambodia, Laos, Vietnam, and Myanmar. *P. falciparum* SNPs will either be genotyped using a high-density Nimblegen DNA microarray or called from short-read sequencing data. Linear mixed models and Random Forests will be used to estimate associations between individual SNPs and parasite clearance half-life, while adjusting for important covariates and taking into account multiple comparisons. Candidate markers of artemisinin resistance identified within high-priority genes located in validated genomic regions will be presented.

## 329

### PREDICTORS OF SEVERE MALARIA IN CHILDREN UNDER FIVE YEARS OF AGE IN BURKINA FASO

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In endemic and sub-Saharan countries with high incidence and death of malaria in children, malaria eradication has been a key objective for many states. Malaria eradication program is a major public health priority in Africa, but finding an effective strategy of fighting is a challenging task. In Burkina Faso, most hospitals face the curse of malaria without access to pediatric intensive cares. Understanding the various factors which contribute to the severity of malaria is very difficult and there is a little information. To assess predictors of severe malaria for children, we assume that the incidence of clinical form of severe malaria is unknown and there are unknown predictors of severe malaria with its consequencies for contributing to increase the mortality. A total of 510 children (mean age 23.5 months) with suspect malaria were drawn from one District and one Regional Hospital of Koudougou from July to September 2012 using the cross sectional study. Each child was screened using blood smear to identify whether he/she had severe malaria using the criteria set by the World Health Organization. When a child is identify as having malaria, either severe or no severe malaria, the main caregiver is interviewed by a trained interviewer using a structure questionnaire. To describe the association between factors and malaria, logistic regression approach was utilized using SPSS 17.0. Out of 510 children, 53,3% were blood smear positive, 35.49% were not able to determine and 11.28% were negative but showed a clinical signs of malaria. 201 (39.4%) were severe malaria. Most of the patients (54.9%) were living in rural area. Of 29 (14.4%) who died with severe malaria, 16.1% died from anemia. Major predictors for severe malaria and its deaths were: rural area (11.6; p=.001); low income (OR=9.6;p=.001); illiteracy (OR=14.8; p=.001); cost of treatment (OR=10.9; p=.001); low hemoglobin (OR=496, p=.001); Hyper*parasitemia* (OR=23.5; p=.001), travel time (OR=4.1; p=.001); and self treatment (OR=2.7; p=.002). These findings are strongly consistent with previous studies in Malawie, Senegal, Cameroon, Zambia and Burkina Faso respectively. We found that severe malaria is still a serious public health concern for young children in resources limited setting. There is need for more health education, reducing cost of treatment, improve socio economic status, improve access to health facilities, and encourage early care seeking.

## 330

### MALARIA DIAGNOSTIC SERVICES AND TREATMENT PRACTICES FOR FEBRILE CHILDREN UNDER 5 YEARS - MAKARFI, NIGERIA

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Malaria is the leading cause of childhood mortality in Nigeria. Averagely, children <5years (U5) are prone to three episodes annually. In 2005, the national malaria policy recommended Artemisinin-based combination therapy (ACT) due to established resistance to Chloroquine (CQ). In 2011, the policy was revised to ensure parasite-based diagnosis before treatment of malaria. However, treatment remains largely presumptive. We conducted a hospital-based cross-sectional study in a low malaria prevalence setting to determine awareness of malaria diagnostic services (MDS) and treatment practices for fever among caregivers of febrile U5 (FU5). We interviewed consecutively selected caregivers of 295 FU5, attending Makarfi General Hospital, Kaduna state, Nigeria; from December 2010 to August 2011. We included all eligible FU5 without rash. Information on factors influencing awareness of MLS and pre-hospital treatment (PHT) was collected. We examined the Giemsa-stained blood smear of FU5 for malaria. Fifteen (5.1%) caregivers have ever heard about MDS. Eleven (3.7%) caregivers were ever offered MDS by physicians. Being formally educated (Prevalence Odds ratio (POR): 0.05, 95% Confidence

Interval (CI): 0.01-0.20), living <5km from a health facility (POR: 4.21, CI: 1.39- 12.55), being a government staff (POR: 9.18, CI: 1.74- 39.93) and ever being offered MDS (POR: 35.09, CI 10.13-134.00) were positively associated with awareness of MDS. Overall, 201(67.9%) children had received any PHT, 121 children (41.0%) at patent medicine stores. Of the 31(10.5%) FU5 diagnosed with malaria and 264 (89.5%) without malaria diagnosis, 13 (41.9%) and 65 (24.6%) had PHT with CQ respectively. Awareness of MDS remains low. Treatment of FU5 against malaria is predominantly inappropriate. There is a need to sensitise caregivers and health staff on use of ACTs and adherence to confirmatory malaria diagnosis.

## 331

### COMBINING DATA FROM MULTIPLE SPATIALLY REFERENCED SURVEYS: GEOSTATISTICAL ANALYSIS OF CHILDHOOD MALARIA IN CHIKHWAWA DISTRICT, MALAWI

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Geostatistical methods are becoming more widely used in epidemiology to analyze spatial variation in disease prevalence. These methods are especially useful In resource-poor settings where disease registries are either non-existent or geographically incomplete, and data on prevalence must be obtained by survey sampling of the population of interest. In order to obtain good geographical coverage of the population, it is often necessary also to combine information from multiple prevalence surveys in order to estimate model parameters and for prevalence mapping. However, simply fitting a single model to the combined data from multiple surveys is inadvisable without testing the implicit assumption that both the underlying process and its realization are common to all of the surveys. We have developed two classes of bivariate generalized linear geostatistical models (GLGM's) to combine data from two spatially referenced surveys so as to address each of two common sources of variation across surveys: variation in prevalence over time; and variation in data-quality. In the case of surveys conducted at different times, we assume an autoregressive dependence between the two components of the bivariate process. In the case of surveys that differ in quality, we assume that one of the surveys provides a gold standard whilst the other is potentially biased. For example, one survey might use a random sampling design, the other an opportunistic convenience sample. For parameter estimation in either models, we have developed a rapid Monte Carlo method for approximate evaluation of the likelihood function. Both approaches can easily be extended to analyze data from more than two surveys. We describe an application to malaria prevalence data from Chikhwawa District, Malawi. The data consist of two Malaria Indicator Surveys (MIS's) and an Easy Access Group (EAG) study, conducted over the period 2010-2012. In the two MIS's, the data were collected by random selection of households in an area of 50 villages within 400 square kilometers, whilst the EAG study enrolled a random selection of children attending the vaccination clinic in Chikhwawa District Hospital. The second sampling strategy is the more economical, but the sampling bias inherent to such "convenience" samples needs to be taken into account.

## 332

### ETHIOPIA, NATIONAL MALARIA INDICATOR SURVEY 2011 (MIS-2011): COVERAGE AND USE OF MAJOR MALARIA CONTROL INTERVENTIONS

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Mmalaria still remains major public health problem in Ethiopia. Malaria Indicator Survey (MIS) had been conducted in 2007 aimed at measuring key malaria interventions coverage and prevalence of malaria morbidity as well as *parasitemia* and under five children anemia. MIS-2011 has been conducted from October to December 2011 to measure the progress of malaria prevention and control efforts undertaken since 2007 and see whether the goals set forth in the FMOH National Strategic Plan for Malaria Prevention and Control 2005 - 2010 were achieved. The survey was a national level study that used a two-stage random cluster sample of 10,444 households in 440 census enumeration areas. A total of 47,248 people participated in the survey. Data were collected using Roll Back Malaria M&E Reference Group household and women's questionnaires, which were adapted to the local context and assisted by PDA. Data collected were transferred in MS access data base and analysed using STATA and SAS statistical soft-wares. The results indicated that 55.2% of households have at least one mosquito net (of any type), and 54.8% of households have at least one long-lasting insecticidal net (LLIN). Of children U5, 38.2% slept under a net the night before the survey, and 64.5% of children U5 slept under a net in a household that owned at least one net. These figures were 35.3% and 63.8% respectively for pregnant women. IRS had been conducted in 46.6% of households in the last 12 months preceding the survey. It was reported that 19.7% of children U5 had suffered from a fever in the two weeks preceding the survey. Of these children, 51.3% sought medical attention within 24 hours of onset of fever; 32.6% took an antimalarial drug and 8.5% took the drug on the day of fever onset. Among the febrile children who were treated with an antimalarial on the day of fever onset, 68.9% sought their treatment from public health facilities. Malaria parasite prevalence in areas <2,000m was 1.3% by microscopy blood-slide examination for all ages, with 1% of these being *Plasmodium falciparum* and 0.3% being *P. vivax.* Compared to the MIS 2007 result, it is observed that net ownership use decline from 65% to 55.8%. However, IRS coverage grew from 20 to 46.6 % . Except some indicators, the findings of the survey show, all in all, the malaria control program in Ethiopia has sustained the gains in malaria control and prevention and the results implicate the right direction of the country in achieving its target.

## 333

### THE EFFECT OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY ON THE INCIDENCE OF MALARIA AND OTHER DISEASES IN CHILDREN LIVING ON THE COAST OF KENYA

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It is widely believed that the prevalence of glucose-6-phosphate dehydrogenase deficiency (G6PDd) in human populations reflects selection by malaria. Nevertheless, few detailed epidemiological studies have investigated the impact of G6PDd on the risk of either malaria or of other common diseases among children living in malaria-endemic areas. Studies that have been conducted have yielded confusing results, one potential explanation being a lack of uniformity regarding the methods used to define G6PDd. We have investigated the impact of G6PDd both on the risk of malaria and other diseases among children living in Kilifi District on the coast of Kenya, where previous studies have shown that the only significant cause of G6PDd is the G6PD A- variant. In a study involving 1332 case-children presenting to Kilifi District Hospital with severe *Plasmodium falciparum* malaria and 7500 controls recruited from within the same geographic area we found that heterozygous females with the G6PD A- allele were significantly protected against severe *P. falciparum* malaria (OR 0.75; 0.61-0.91; p=0.004). Protection was also seen among homozygous females, although significance was lost on adjusting for confounding factors (0.70; 0.39-1.23; p=0.22). No protection was seen among hemizygous males (OR 1.06; 0.43-1.11; p=0.135) who, to the contrary, showed an increased risk of severe malaria anaemia (1.80; 1.24-2.61; p=0.002). In a cohort study conducted in the same geographic

area we found no effect of the G6PD A- allele on the incidence of either uncomplicated *P. falciparum* malaria or that of any other common childhood diseases with the exception of undiagnosed febrile illnesses, which occurred significantly less frequently among heterozygous females (IRR 0.61; 0.41-0.92; p=0.02). *P. falciparum* parasite densities were lower in G6PD A- carriers than normal children during episodes of clinical malaria but this only reached significance among homozygous females with uncomplicated disease. Through a range of studies conducted in an area where the G6PD A- variant is the only major cause of G6PDd we find that protection from severe malaria is limited to heterozygous females while hemizygous males are significantly predisposed to severe malaria anaemia. Reduced parasite densities in G6PD A- carriers are consistent with a mechanism involving enhanced clearance of *P. falciparum* -infected G6PDd red blood cells.

## 334

### INCREASING INCIDENCE OF *PLASMODIUM KNOWLESI* MALARIA FOLLOWING CONTROL OF *P. FALCIPARUM* AND *P. VIVAX* MALARIA IN SABAH, MALAYSIA

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The simian parasite *Plasmodium knowlesi* is a common cause of human malaria in Malaysian Borneo and threatens the prospect of malaria elimination. However, little is known about the emergence of *P. knowlesi*, particularly in Sabah. We reviewed Sabah Department of Health records to investigate the trend of each malaria species over time. Reporting of microscopy-diagnosed malaria cases in Sabah is mandatory. We reviewed all available Department of Health malaria notification records from 1992-2012. Notifications of *P. malariae* and *P. knowlesi* were considered as a single group due to microscopic near-identity. From 1992-2012 total malaria notifications decreased dramatically, with *P. falciparum* peaking at 33,153 in 1994 and decreasing 46-fold to 716 in 2012, and *P. vivax* peaking at 15,857 in 1995 and decreasing 33-fold to 478 in 2012. Notifications of *P. malariae /P. knowlesi* also demonstrated a peak in the mid-1990s (614 in 1994) before decreasing to ≈100/year in the late 1990s/early 2000s. However, *P. malariae /P. knowlesi* notifications increased >10-fold between 2004 (n=59) and 2012 (n=815). In 1992 *P. falciparum* , *P. vivax* and *P. malariae /P. knowlesi* monoinfections accounted for 70%, 24% and 1% respectively of malaria notifications, compared to 36%, 24% and 40% in 2012. The increase in *P. malariae /P. knowlesi* notifications occurred state-wide, appearing to have begun in the southwest and progressed north-easterly. We conclude that a significant recent increase has occurred in *P. knowlesi* notifications following reduced transmission of the human *Plasmodium* species, and this trend threatens malaria elimination. Determination of transmission dynamics and risk factors for *knowlesi* malaria is required to guide measures to control this rising incidence.

## 335

### DATA AND MODELS TO QUANTIFYING THE ROLE OF HUMAN TRAVEL IN MALARIA EPIDEMIOLOGY

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Human movements contribute to the transmission of malaria worldwide. Identifying sources and sinks of imported infections due to human travel and locating high-risk sites of parasite importation could greatly improve public health control programs. Here, we use spatially explicit mobile phone data and simulated disease models to identify the dynamics of human carriers of pathogens between regions. We address a number of issues with modeling human travel for malaria control and available data sources.

## 336

### A DEEPER INSIGHT INTO IDENTIFYING MALARIA HOTSPOTS: TOPOGRAPHY, CLIMATE AND GENOTYPE STRUCTURE

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A microgeographic scale study is conducted in a 200×150 km2 area of western Kenya to investigate the impacts of environmental, spatial, and genetic factors on malaria prevalence. Approximately 13,000 samples collected in 2011-2012 representing 47 sites in western Kenya were examined. PCR- and microscopy-based measures indicate a strong and inverse correlation between elevation and prevalence rate. In addition, temperature, evapotranspiration rate, and wind speed are significantly related to prevalence. When all variables are included in the principal component analyses, we found that areas of relatively lower prevalence (<5%) occupy the greatest range of environmental amplitude compared to areas of higher prevalence (>25%). This suggests that topography and climate are generally more homogeneous in areas where malaria is prevalent and endemic. Geostatistic analyses using prevalence data indicate that the number and position of sampling points influence both spatial structure of prevalence and risk predictability within the studied area. The number of predicted malaria hotspot decreases when sampling points are reduced systematically in each of the iterations. This demonstrates that the detectability and precision of malaria hotspots are highly dependent of the number of sites being examined. Further, we utilize Fluidigm technology to obtain SNP genotypes of malaria parasite across the studied sites. A total of 72 SNPs representing both synonymous and non-synonymous changes in the exons and introns of various genes, and intergenic regions of the *Plasmodium falciparum* genome were assessed by the Fluidigm EP1 system and dynamic arrays. These SNPs are shown to provide a genetic signature of individuals that allow us to identify polyclonal samples as well as to scrutinize genotypic distribution among populations. Findings of environmental, spatial, and genetic analyses altogether shed light on factors and patterns of malaria transmission.

## 337

### RELATIVE CONTRIBUTION OF *BACTEREMIA* AND MALARIA TO UNEXPLAINED ACUTE UNEXPLAINED FEVER IN UNDER-FIVE CHILDREN IN RURAL WESTERN KENYA

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Malaria and *bacteremia* are common causes of under-five febrile illness and mortality in sub-Saharan Africa. The two conditions are clinically indistinguishable and, while rapid diagnostic tests for malaria are now widely available in many settings, microbiologic laboratories to diagnose

*bacteremia* are very limited. Therefore studies of factors that can inform differential diagnosis of malaria and *bacteremia* in resource poor settings are critical to the appropriate management of febrile children and rational use of antimalarials and antibiotics. We determined the relative prevalence and predictors of malaria and *bacteremia* among febrile (axillary temperature ≥ 37.5°C) children consecutively presenting at two regional rural hospitals in Western Kenya. We collected detailed demographic, anthropometric, medical and clinical examination information from consenting children aged 6-59 months. Malaria positivity was determined by smear microscopy or rapid test. We performed blood cultures, isolate identification and antibiotic susceptibility testing using BACTEC™ 9050 and MicroScan Walkaway40® systems. All children were also tested for HIV. We studied 546 children of which 91 (16.5%) had malaria and, 20 (3.7%) had clinically significant *bacteremia*. Only 6 (1.1%) children were co-infected with malaria and *bacteremia*. Among children with *bacteremia*, *salmonella* strains were the most common isolates identified (12/20, [60%]). Factors significantly associated with malaria in multivariate analysis included being >2y old (OR=2.72; 95% CI: 1.45-5.08), presence of ≥1 WHO-integrated management of childhood illness (IMCI) danger signs (OR=4.11; 95% CI: 2.28-7.41), and consumption of unsafe water (OR=2.52; 95% CI: 1.26-5.03). We found no association between *bacteremia* and risk factors such as HIV, malnutrition, use of unsafe water, or presence of a WHO-IMCI danger sign. Unlike *bacteremia*, malaria is common among febrile children in rural Western Kenya, especially among those older than 2 years, manifesting IMCI danger signs, and consuming untreated water.

## 338

### THE MIGRATORY PATTERNS OF *PLASMODIUM FALCIPARUM* MALARIA PARASITES BETWEEN ISLANDS OF THE LAKE VICTORIA REGION, KENYA

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Malaria is responsible for extensive mortality and morbidity with the current statistics recording approximately 660,000 deaths and 220 million malaria cases globally in 2010.Today, 11 countries in Africa have embarked on malaria elimination. Although the continent is witnessing an epidemiological transition with plummeting malaria risks, the feasibility of malaria elimination in settings of high transmission tropical Africa remains unclear.A molecular epidemiological survey aimed at generating baseline data in a malaria endemic site to reveal patterns of disease transmission across the Lake Victoria islands is currently ongoing. The contribution of inter-island human, parasite and mosquito migration to the maintenance of malaria transmission will be investigated for the effective planning of malaria interventions. Following the application of malaria control measures, the likelihood of malaria reappearing due to reintroduction of parasites from outside the control region can be assessed. In addition, reductions in malaria endemicity may lead to the emergence of clusters or foci of malaria transmission. These can also be identified and control measures scaled up for effective and sustained malaria elimination. *Plasmodium falciparum* isolates were collected from various locations on the islands and the surrounding mainland, and parasites DNA extracted. The samples were then genotyped using eight genome-wide microsatellite markers. Population genetics analyses were then performed in order to investigate the population dynamics of parasites from the study area with emphasis on determining whether there are distinct foci of transmission, or whether the population is in panmixia. . Further genotyping was performed on *P. falciparum* merozoite surface proteins 1 and 2 (Pfmsp1 and Pfmsp2) to evaluate the degree of multiplicity of infection in each region. We will present the results of this parasite population profiling, and discuss the implications of our findings for the efforts to eliminate malaria from this region.

## 339

### TREND OF MALARIA MORBIDITY IN KERSA, SOUTHWEST ETHIOPIA

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Malaria is a highly infectious disease, causing the major cause of disease and death in sub-Saharan Africa, especially among children and pregnant women. This paper assessed the trend of malaria cases from September 2005 to August 2011. A retrospective analysis of daily outpatient consultation records was obtained from Jimma Health Bureau. Moreover, One year cross-sectional blood film examination was performed in Bulbul, Serbo and Bala Wajo health centers in Southwest Ethiopia.Data were entered and checked, thereafter analyses were performed using excel and SPSS version 16 software. Descriptive statistics was used to assess the trend of malaria cases detected over six year period. We assessed 6 years trend of malaria case in Kersa area between September 2005 to August 2011. A total of 57482 malaria cases were diagnosed in the three Health centers. Among these, a total of 15865 were children under five years of age. The majority (88.80%) of malaria cases were reported in 2006/2007. Moreover, the percentage of *Plasmodium falciparum* and *P. vivax* were *57.37%* and 42.63% respectively. To minimize reliability and validity of secondary data, one year cross sectional analysis was performed in Kersa Woreda from three health centers. Concerning the one year cross-sectional study, males were more affected (60.1%) than females (39.9%). In the same year 33.8% of the positive cases were children. The proportion of malaria cases detected among clinical suspects over the 5 year period was 51.13% .On the other hand the proportion of malaria cases was during one year blood film examination was 25.32%. Despite recent decline in malaria consultation rates, malaria was a problem in Kersa. Furthermore results presented in this study suggest that the burden of malaria in children <5 years of age is still significant. Our assessment indicated that annually, malaria consultations peaked during September to December which coincides with the end of the rainy season.

## 340

### *PLASMODIUM KNOWLESI* TRANSMISSION IN ANDAMAN AND NICOBAR ISLANDS OF INDIA AND DRUG RESISTANCE GENOTYPES

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Recent studies in Southeast Asia have shown zoonotic transmission of *Plasmodium knowlesi* to humans. It was not known whether *P. knowlesi* transmission occur in India. Recently, we investigated the possible presence of *P. knowlesi* in Andaman and Nicobar group of Islands as these islands are situated in the Bay of Bengal in an ecological zone similar to Southeast Asian countries such as Malaysia and Indonesia where transmission of this parasite has been shown. This was a retrospective investigation of clinical samples obtained from previous studies involving several islands in this region. In this investigation we sequenced the chloroquine resistance transporter(CRT) and dihydrofolatereductose (DHFR) genes of *P. knowlesi* and other *Plasmodium* species. The merozoite surface protein-1 and 18S rRNA genes of *P. knowlesi* were also sequenced from these samples. Among 445 samples analysed , only 53 of them had P. *knowlesi*-specific gene sequences.While 3 of the (86.79%,n=46)or *P. vivax* (7.55%,n=4) but none with *P. malariae* or *P. ovale* . All *P. knowlesi* isolates contained wild type sequences of crt and dhfr genes while *P. falciparum* isolates had mutation in CRT and DHFR marker genes. The mutation pattern indicates that the same patient having a mixed infection may be harbouring the drug susceptible *P. knowlesi* parasite and a highly drug resistant *P. falciparum* parasite. The implications of these findings in the context of evolving drug resistance and treatment strategies including ecological changes associated with transmission of *P. knowlesi* will be discussed.

## 341

### POPULATION STRUCTURE OF *PLASMODIUM VIVAX* IN AN URBAN VILLAGE OF THE PERUVIAN AMAZON (SAN JUAN-IQUITOS)

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In Latin America, Peru is among the countries with the highest malaria burden, mainly due to *Plasmodium vivax* infections. However little is known about *P. vivax* genetic structure, which is essential to describe the transmission dynamics, in the Peruvian Amazon where most of the malaria cases occur. Hereby we determined the genetic diversity and population structure of *P. vivax* isolates collected in the Peruvian Amazon. A total of 65 *P.vivax* patients recruited in San Juan city (Iquitos, Peru) between April, 2008 and February, 2009 were treated radically with chloroquine and primaquine and followed up monthly for 2 years with systematic blood sampling. All samples were screened for malaria parasites by microscopy and PCR, subsequently all *P. vivax* infections were genotyped using 15 microsatellites. Parasite population structure and dynamics were determined by computing different genetic indices. *P. vivax* population structure was determined by multilocus genotyping using 15 microsatellites on 297 *P. vivax* infected blood samples collected. The genetic diversity was determined by calculating the expected heterozygosity (He). Both linkage disequilibrium and the genetic differentiation were estimated. The population characteristics were assessed only in samples with monoclonal infections (n=242). The proportion of polyclonal infections was 10.7%. The *P. vivax* populations circulating in San Juan city are genetically diverse however they have a low recombination rate. Clonal parasite reproductive events in this area are indicated by the presence of significant linkage disequilibrium. The low malaria transmission may favor as well the clonal parasite population.

## 342

### EXPLORATION MALARIA AND OTHER VECTOR-BORNE ILLNESSES INCIDENCE IN ENTOMOLOGICAL WORKERS

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Mosquito collections are vital to surveillance certain vector-borne illnesses (VBI). Human-baited collections (HLC) remain the only reliable method to assess the presence and behavior of anthropophilic malaria vectors. However, HLC is considered to increase the risk of acquiring malaria, although empirical evidence from low-endemic settings is lacking. We explored perceived and actual risks associated with HLC in the Peruvian Amazon Basin, a low-endemic malaria setting, through a longitudinal study of entomological workers (EW) from Iquitos. Malaria/VBI incidence and exposure were determined every three months by microscopy, nested PCR and enzyme-linked immunosorbent assay. Signs/symptoms, work-conducted practices, and behaviors were assessed monthly. A focus group was conducted to understand the knowledge and perceptions of EW-associated risks. We enrolled 19 EW (males=89%, mean age=37 years, mean EW time=9 years), who qualitatively identified themselves as study personnel. Their HLC-related self-perceived risks were: VBI, rabies, dog/sneak bites, traffic accidents, and working in narco-terrorism affected areas. HLC was carried out by 84% (n=16) of EW, 61% as their regular activities. The self-reported lifetime prevalence of infectious diseases was: malaria (68%), diarrhea (68%), dengue (53%), leishmaniasis (5%), hepatitis (5%), and Oropouche fever (6%). Protective measures used were: long pants (97%) and sleeves (72%), mosquito repellent (20%), head nets (20%) and insecticide-impregnated clothing (5%). All study participants were malaria-free at baseline, 15% exhibited circulating IgG antibodies to PvMSP-1, 84% to dengue virus, and 21% to other arboviruses. Four participants had malaria over the 10 months of study (169 person-months) representing an incidence of 2.3 cases per 100 person-months in this population. All malaria free individuals (n=15) remained seronegative to PvMSP-$1_{19}$ from baseline until the end of follow-up. Out of 86 HLC-months, only 5% resulted in malaria infections. EW are a heterogeneous group with diverse behaviors and preventive practices. These findings indicate that additional studies need to be conducted with and adequate comparison group to determine if HLC puts EW at increased risk for malaria/VBI even in low endemicity settings. As research staff, EW should be provided with proper education and occupational health support to minimize any VBI risks.

## 343

### TRANSMISSION OF MALARIA: EPIDEMIOLOGICAL AND CLINICAL PROFILE OF SEVERE MALARIA IN ADULTS IN KINSHASA (2007-2012)

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Malaria is endemic in the tropics. The age group most exposed to severe forms is that of children aged 0 to 5 years. Due to natural immunity, adults are protected and rarely develop serious forms of malaria. Nowadays, more and more cases of adults in the severe form of malaria are reported. The objective of our study was to describe epidemiological and clinical profile of severe malaria in adults in Kinshasa. 148 cases from 9757 patients admitted to infectious disease clinic or 1.5% of all cases of hospitalization showed the severe form of the malaria. The female sex was predominant with a sex ratio of 1.06. The age group most struck was that of 18-34 years (35.8%). Fever was present in 64.9% of patients and pale mucous membranes and hemoglobinuria were respectively found in 12.2% and 4.1% of cases. The *parasitemia* was observed in 44% of patients. Cerebral malaria has been reported in 71.4% of cases. Quinine intravenous and intramuscular artemether were administered respectively in 95.5% and 4.1% of cases the outcome was favorable in 130 patients (87.8%). There were 18 deaths (12.2%). In conclusion, although rare (1.5%), severe malaria remains a reality in Kinshasa and should be described to take effective control measures.

## 344

### NOVEL SEROLOGIC ASSAYS OF *PLASMODIUM FALCIPARUM* EXPOSURE

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Assessing *Plasmodium falciparum* (Pf) exposure is labor intensive and inaccurate. Serologic assays offer promise of greater precision at low cost, but appropriate antigens against which to assess responses representative of exposure are uncertain. Kinetics of Pf-specific antibody responses differ by antigen, suggesting that appropriately selecting antigens for antibody assessment will increase accuracy of Pf exposure estimations. Analysis of Pf protein microarrays probed with plasma from Malian children, aged 2-10 years, identified antibody kinetics predicting cumulative and recent

exposure. Based on these analyses, we probed a smaller array with plasma collected at 4 years of age from 79 children in Tororo, Uganda, where malaria transmission intensity is very high (entomological inoculation rate >300). Subjects had been followed from ≤10 months of age with continuous passive surveillance and monthly blood smears. Cumulative exposure markers were identified by linear fit of antibody intensity vs. total number of malaria episodes. Recent exposure markers were those that best predicted time since last *parasitemia*, assuming exponential antibody decay. Of 39 cumulative and 34 recent exposure markers selected in Malian children, 20.5% and 32.4% respectively were also predictive of exposure in Ugandan children. Cross-sectional analysis of responses to erythrocyte stage antigens AMA1 and MSP1 are commonly used to assess Pf exposure. However, in our age-restricted cohort, the highest R2 for the 13 AMA1 and MSP1 antigen fragments on our array was 0.08. In contrast, PfEMP1, MSP11, and ETRAMP4 R2 ranged from 0.34-0.40. Evaluated together, these 3 antigens gave a cumulative exposure R2 of 0.52. Serologic responses to ACS5 and ETRAMP4 predicted time since the last parasitemic episode with an R2 of 0.65, allowing estimation of recent exposure. Though this new approach toward predicting Pf exposure demonstrates some variance for individuals, it offers promise for precise population estimates. We are currently evaluating responses across multiple transmission settings and age ranges in Ugandan children to further generalize these findings and to develop robust models of Pf exposure.

## 345

### MALARIA MORBIDITY AND PREVALENCE OF INTESTINAL PARASITES DURING THE FIRST YEAR OF LIFE IN RURAL SENEGAL

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Malaria, the first parasitic endemic disease throughout the world, touches especially in Africa in the south of the Sahara, the children of less than five years. In Senegal, the various fight plans, involved a clear reduction among case of malaria which passed from 408,588 to 30,800 between 2006 and 2009 in the children of less than five years. Thus in front of the frequency of malaria, anaemia and the intestinal parasitic bearing in the children of less than 5 years, we proposed more specifically to study them in the infants up to one year where data are fewer. We conducted a longitudinal prospective descriptive study of follow-up of a cohort of children since the age from 4 to 6 weeks during one year. The follow-up visits were monthly. The subjects of the study were also visited in a weekly way in residence for early detection of the cases of malaria. The rate of haemoglobin was measured with a device Hemocue Hb 301. An examination of saddles in a fresh state was carried out .The study protocol was subjected and approved as a preliminary by the national ethics committee of Senegal. At the end of one year follow up visits for participants(from November 2010 to March 2012), one clinical malaria at 59 days of age confirmed by finger prick thick (1640 Pf/µl) was observed. The prevalence of malaria in our study was 0.7% (1/138) by taking account of the 12 withdrawals of study. No case of malaria infection was noted. The mean haemoglobin was 12.4 mg/dl at inclusion. The moderate anaemia was 16.7% at inclusion and had appreciably increased to 55.3% at 6months and 51.9% at 12 months. The prevalence of the bearing for the principal intestinal parasitic species found in our study is the following one by order of importance: *Giardia intestinalis* (15.4%), *Ascaris lumbricoides* (8.5%), *Entamoeba coli* (1.5%), yeasts (1.5%), *Trichuris trichiura* (0.8%). In our cohort of study the acute respiratory infections dominated in the reasons for consultation. Indeed 94.6% of the children came to consult at least once for an acute respiratory infection. Two deaths were noted during follow up for breath infections. Preliminary results of this study confirm that infants are protected during their first months of life. These results were reinforced because all participants of the study were followed after the period of intensive transmission between September and November.

## 346

### MALARIA TREATMENT SEEKING BEHAVIOR: FACTORS INFLUENCING CARE SEEKING FROM SIMILAR/EQUAL LEVEL, RURAL AND MORE DISTANT FACILITIES

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Distance greatly affects health care access. In rural, high malaria endemic Uganda (562 infective bites per person per year) with an unreliable transport system, some patients walk, cycle, or ride pillion on a motorbike, to more distant lower level government run health facilities (PHF) for care. In this study we explore the main factors compelling caregivers and/or patients to travel to further PHF for care and thus, provide a framework for improvement of patient centered care advantaged by proximity. We collected standard HMIS out patient data (OPD) from20 PHF in a cluster randomized trial with 10 in the health facility intervention (HFI) and 10 in standard care (SC), in 7 sub-counties of Tororo district. OPD was collected from Apr-2011 to date and we conducted spatial analysis on 14 months of the data to examine patients' residence in relation to the PHF they visited. 263,873 patients with mean age of 17.9yrs were seen, 31.6% being under-fives. We defined distance traveled as the Euclidean distance -centroid of a patient's village of residence to the PHF visited- categorized as: <2km; and, >2km. We quantitatively analyzed OPD for influence of: level of PHF, Intervention arm, age, gender, malaria diagnosis, and recorded fever history. Also, qualitative data in the study was analyzed for patients' motivations in choosing PHF. Preliminary results suggest: 40.4% of patients travel >2Km to a PHF; odds in traveling >2Km for HFI to be 22% higher than for SC, adjusting for health facility level, gender, malaria diagnosis and age; odds for under-fives are 8% higher than for older patients, adjusting for other factors; odds for patients with no malaria diagnosis are 5% higher than for those with a malaria diagnosis, adjusting for the other factors; and, No statistical difference between male or female, or having a history of fever or not. Testing services were cited as a major reason to travel further. We posit this explains traveling further to HFI -offering malaria RDT testing- versus SC. We will present full results with the full data and other dynamics over time.

## 347

### THE EFFECTS OF URBANIZATION ON MALARIA METRICS IN UGANDA

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Sub-Saharan Africa is expected to show the greatest rates of urbanization over the next 50 years. Urbanization has shown a substantial impact in reducing malaria transmission, because, amongst other factors, urban areas generally provide unfavorable habitats for *Anopheles*, and urban populations are generally healthier and have better access to healthcare. Statistical relationships have been explored at global and local scales, but only examining the effects of urbanization as a binary variable, and only looking at the effects of urbanization on a single malaria metric. Here we undertake the first analysis that examines the impact of varying degrees of urbanization on a variety of malaria metrics. Cohorts of 100 households (HH) across three contrasting districts of Uganda (Jinja, Tororo and Kanungu) were followed for a 11/2 year period. For each district and HH, *Anopheles* catches were measured, Entomological Inoculation Rates (EIR) were calculated, parasite rates (PR) were measured and clinical metrics were gathered. Measurements of the intensity of urbanization in the vicinity of each HH were also calculated through HH density and satellite imagery based metrics. Correlation analyses between the variety of urban intensity and malaria metrics were then undertaken. Consistent and significant correlations between urban intensities and malaria metrics were found. HH density and urban land cover proportion measured through

satellite imagery classification both showed significant correlations with decreased numbers of *Anopheles*, EIR, PR and clinical incidence. These differences were much clearer for Jinja district, which showed the greatest range in levels of urbanization of the three studied. These results highlight the substantial impact of urbanization on malaria. With reduced numbers of mosquitoes, lower prevalence and lower clinical incidences, urban areas are shown to have a consistent impact on several malaria metrics. With increasing urbanization rates, results here point to continued declines in rates of malaria across Africa.

## 348

### DECREASED OCCURRENCE OF HIGHLAND MALARIA AFTER INTRODUCTION OF POINT-OF-CARE RAPID DIAGNOSTIC TESTS IN KENYAN HIGHLAND NEAR ELDORET IN 2250 METERS ABOVE SEA LEVEL

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Malaria is known to infect people also in altitudes higher than 2000 m above sea level (a.s.l). Several cases of *falciparum* malaria have been already described from Ethiopian and Kenyan highlands in 2200 m a*.s.l.* Rapid diagnostic tests (RDT) to confirm microscopically positive cases or even to diagnose malaria without microscopy has been shown to be highly sensitive, enough to diagnose malaria and to initiate necessary semi- empiric therapy of slide-positive fever. Eldoret is lying in Cherangani Hills in high of 2200 - 2290 m a*.s.l.* and Ladislaus Batthyanyi -Strattmann Clinic which is located there serves medical services for urban population of around 100000 people and it treats 25000 - 30000 patients per year. The aim of this study was to differentiate if slide-positive cases of fever in altitude of Eldoret highlands (2250 - 2500 m a*.s.l.*) can be confirmed with RDTs as highland malaria. Annual occurrence of malaria has been compared in two periods: (i) before introduction of RDTs (2009-2010) and (ii) after (2011-2012) RDTs have been introduced in combination with microscopy for suspected malaria, especially in patients with travel history to Kenyan down country.From January 2009 to May 2012, 16181 cases of microscopically positive malaria have been diagnosed. In 2009, 7760 cases and in 2010, 5006 cases were diagnosed microscopically. When RDTs were introduced in January 2011, in combination with blood smear microscopy in our laboratory, the number of confirmed cases dropped to 1919 in 2011 and 748 in first six months of 2012, which is 2,5 to 3-times (80%) less than in previous years. In this study, we proved that introduction of point-of-care RDTs into malaria diagnostics in combination with blood smear microscopy led to significant decrease of highland malaria diagnosis as well as artemisinin-based combinational therapy mis-dosing in Kenya.

## 349

### HIGHLAND MALARIA IS NOT RARE BUT SHOWS DECREASING TENDENCY: FOUR YEARS OF FOLLOW UP IN MURAGO HOSPITAL, BURUNDI

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Highland malaria used to be an underestimated entity, however now we know that malaria due to global warming exists also in areas higher than 2000 meters above sea level (a.s.l.). We have seen number of cases of highland malaria in Rwanda, Burundi, Kenya and even in Murago located above 2500 above sea level (a.s.l.). In this study, we have investigated incidence of malaria in Eldoret (2200 meters a.s.l.), Kenya, during four years 2009 - 2012. Diagnosis of malaria was assumed according to clinical signs, blood smear microscopy and positivity of rapid diagnostic test (RDT). We have observed decreasing number of malaria cases at St. Ladislaus Strattmann Clinic, as 7760 patients in 2009, 5006 patients in 2010, but only 1919 patients in 2011 and 1210 patients in 2012 were diagnosed there with highland malaria. Explanation for decreasing number of highland malaria cases may be decline of travel to down country which is probably result of economic crisis. Hereby, we can debate about influence of another economical factor on spread of malaria.

## 350

### A SET OF NOVEL POLYMORPHIC TANDEM REPEATS MAKERS FOR *PLASMODIUM VIVAX*

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Enhanced understanding of the transmission dynamics and population genetics for *Plasmodium vivax* is crucial in predicting the emergence and spread of novel parasite phenotypes with major public health implications, such as new relapsing patterns, drug resistance and increased virulence. Suitable molecular markers are required for these population genetic studies. Here, we focus on the variable number of tandem repeats (VNTRs) which provides valuable information about both the functional and evolutionary aspects of genetic diversity. Although polymorphic microsatellite markers had been optimized in *P. vivax* and used to analyze the population structures of *P. vivax* in many countries, the VNTR based markers and polymorphism have not been examined. In this study, we optimized a set of VNTR markers from 253 *P. vivax* ORF genes and analyzed by Sequence-based Estimation of Repeat Variability (SEVR, analyze DNA sequence and provide "VAR" score). The top 6 of them were used to screen 83 parasite isolates from Southeast Asian countries (China, Korea and Thailand). The total number of alleles per locus ranged between 4 and 9, and the mean of expected heterozygosity (HE) in China, Korea and Thailand are 0.54, 0.51 and 0.72, respectively. Significant linkage disequilibrium was maintained. Population structure showed strong clustering of outbreak isolates from central China and South Korea was observed. Results showed that the genetic variability of 3 populations using these 6 TR makers was similar to those previously reported markers. Furthermore, population structure investigation by using these TR makers has revealed that Chinese (central) and South Korean have similar population structure which was clear different from Thai. We deduce that these molecular markers can be used to characterize the population structure of *P. vivax* in other endemic areas.

## 351

### FINE MAPPING OF THE CD36 BINDING SITE FOR *PLASMODIUM FALCIPARUM* PARASITIZED ERYTHROCYTES

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CD36 is a conserved scavenger receptor that interacts with a diverse number of ligands including *P. falciparum* parasitized erythrocytes (PEs) and oxidized LDL (oxLDL). The crystal structure of LIMPII (Lysosomal

Integral Membrane Protein II), a Class B Scavenger protein closely related to CD36, has recently been solved. Using this structure as a template, the 3D structure of human CD36 (hCD36) was modeled and predicted to exist as a homodimer (dimerization region amino acids 151-181). To test these predictions several mutations were introduced in order to break this secondary structure. The constructs were engineered as GFP fusions and transiently transfected into HeLa or COS-7 cell lines. Transfected constructs were tested for binding of oxLDL and PEs. Binding was quantified and normalized by enumerating only GFP expressing cells. Surface expression of the constructs was determined by immunofluorescence using an anti-hCD36 antibody and a proteinase K protection assay of intracellular proteins. Mutations introduced in this region abrogated binding of both ligands. Residues L158, L161 and in combination K164/K166 seemed to be crucial for maintaining the integrity of the binding site supporting the homodimer model theory. It has been believed that the CD36 binding site for PEs is similar to oxLDL. By studying the binding affinity of different highly conserved CD36 orthologs we found that bovine CD36 does not bind PEs but does bind oxLDL. Interestingly, residues L158, L161, K164 and K166 are highly conserved across orthologs. We hypothesized that a discrete highly polymorphic overlapping region (aa 146-156) might encode the observed differential PE binding. Here we show through detailed site directed mutational analysis that the binding sites for PEs and oxLDL are distinct. Further, ortholog swap analysis between bovine and hCD36 is being used to fine map the PE binding site by defining the minimal requirements to confer PE binding to bovine CD36 and conversely abrogate PE binding by hCD36, while preserving oxLDL binding.

## 352

### CHARACTERIZATION AND ANTIGENICITY OF *PLASMODIUM VIVAX* RHOPTRY-ASSOCIATED LEUCINE ZIPPER-LIKE PROTEIN 1 (PVRALP1), A NOVEL RHOPTRY NECK PROTEIN

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Rhoptry secreted proteins are associated with tight junction and parasitophorous vacuole formation during invasion of host targets cells and are sorted within rhoptry neck or bulb. We have identified blood-stage antigens of *Plasmodium vivax* likely to be highly immunogenic. Of these candidates, a novel protein PVX_096245, which is the ortholog of rhoptry-associated leucine zipper-like protein 1 (RALP1) from *P. falciparum*, was gained for detailed characterization. PvRALP1 contains a novel glutamate(Glu)/glycine(Gly)-rich domain that is conserved in other *Plasmodium* species. In present study, full-length without signal peptide (Ecto) as well as a Glu/Gly-rich domain (Tr) of recombinant PvRALP1 were expressed by using cell-free expression system. Sera screening experiments indicate that PvPvRALP1-Ecto and PvRALP1-Tr possess 58.9% and 55.4% in sensitivity and 95.0% and 92.5% in specificity. The PvRALP1 is localized in rhoptry neck; an apical organelle of the merozoite, and the localization of this protein is firstly defined in *P. vivax* . Of PvRALP1 immunogenicity, cytophilic antibodies were produced simultaneously. The present study suggests that PvRALP1 is immunogenic in humans during parasite infection and it may be a novel potential vaccine candidate in the blood stage of *vivax* parasite.

## 353

### PATTERNS OF GENE FLOW IN *PLASMODIUM VIVAX* POPULATION CURRENTLY CIRCULATING IN SRI LANKA - A COMPARATIVE GENETIC POPULATION BASED STUDY

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Epidemiological evidence of relatively unstable and low intensity malaria transmission due to successful elimination strategies lead Sri Lanka to achieve malaria pre-elimination status in the year 2008. Understanding the population genetic structure of current and previous local *Plasmodium vivax* isolates is important to (i) examine the degree of genetic isolation of these populations, and (ii) ascertain whether subsequent outbreaks would be due to residual transmission or due to introduction of new parasite strains to the parasite population, enabling population specific malaria control measures Sequences of *P. vivax* isolates that circulated locally, a decade ago was obtained from the Genebank (pvmsp3α: N=17; pvdbpII=100, pvmsp142= 95, pvcsp: N=60). PCR amplification and sequence analyses of these four polymorphic *loci* of *P. vivax* were carried out using 16 samples collected recently (2011-2012). Expected heterozygocity (He) and the genetic differentiation (Fst) was examined using DNasp 5.1 software, to draw comparison of current and previous population genetic structures. Low mean (He) in the current *P. vivax* population (He=0.76) compared with the previous population (He=0.93) was observed for all four genes indicating less gene diversity in the currently circulating isolates. However, the He of pvmsp-142 (0.962) current population was higher than that of the previous (0.978). Genetic differentiation (Fst) between the two test populations was highest in pvmsp3α (0.20719) followed by pvcsp (0.1271) indicating great differentiation between the two populations. PvdbpII (0.0793) and pvmsp-142 (0.0018) showed moderate and little genetic differentiations, respectively. Linkage disequilibrium was maintained across the current population except for pvmsp3α. A reasonable degree of overlap of amino acid haplotypes in these four proteins and not many novel a.a haplotypes were observed between current and previous populations. Thus these results for the first time in Sri Lanka suggest that new *P. vivax* variants may have been introduced to the island with simultaneous residual transmission of previously detected alleles. Further investigation is needed in order to ascertain the risk of re-introduction.

## 354

### HIGH RESOLUTION MELTING: AN ADAPTABLE AND DEPLOYABLE METHOD IN THE FIELD TO MONITOR *PLASMODIUM FALCIPARUM* GENETIC POLYMORPHISMS

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Understanding the genetic variation in malaria parasite populations and how selective pressures such as drug treatment regimes alter these patterns of genetic variation can be used to identify molecules responsible for changes in drug response, and to develop tools that can provide an early warning system for the emergence of drug resistance when new anti-malarial drug pressure is applied. These tools should be fast, sensitive, unambiguous, and cost-effective, and deployable in malaria endemic field sites. We have successfully deployed High Resolution Melting (HRM) technique in Senegal to analyze molecular four genes pfcrtK76T,

pfmdr184/86/1042, dhfr51/59/108/164 and dhps437/540/581/613 in 27 parasite samples from ACT *in vivo* efficacy study performed in Thies, Senegal. The prevalence of pfcrtK76T mutant and mixed alleles are: 33.33% and 11.11% mixed; 96.30% and 3.70% mixed for dhfrN51I; dhfrC59R (92.6% and 3.70%); 92.59% and 7.41% for dhfr108N; and 100% wild type dhfr164I. For the dhps gene we have 100% wild type in *loci* A581G and K540E; 55.56% mutant and 11.11% mixed for A437G; 3.70% mutant allele in *loci* A613T/S. For pfmdr, all parasites were wild type at N1042D, but in *loci* Y184F we found 55.6% mutant and 3.7% new sequence; 88.9% wild type and 11.1% mutant for the *loci* N86Y. Our results illustrate that the HRM is an adapted and deployable method in malaria endemic countries to track parasite genetic polymorphisms in real time.

## 355

### EPIGENETIC CHANGES IN THE DEVELOPMENT OF CHLOROQUINE RESISTANT PHENOTYPES OF *PLASMODIUM FALCIPARUM*

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The current scope of chemotherapy in the treatment of malaria has been limited due to the development of drug resistance to a number of highly effective drugs and combinations including chloroquine (CQ). Mutations in the *Plasmodium falciparum* gene *loci* Pfmdr1-N86Y and Pfcrt-K76T are known to confer drug resistance to CQ associated with long term use. However, variable phenotypic effects that mediate drug sensitivity reversal can be caused by epigenetic mechanisms via chromatin remodeling and DNA methylation that affects the state of gene activation and silencing thereby altering the levels of gene expression. In this investigation, we seek to identify the epigenetic changes that mediate altered drug resistance levels to chloroquine. This will involve *in vitro* stepwise selection of *P. falciparum* strains exposed to different concentrations of CQ and assessing for reduced drug sensitivity via a SYBR Green I-based *in vitro* $IC_{50}$ drug sensitivity assay. Variations in gene copy number will be validated via qReal Time-RT PCR for Pfmdr1 while gene mutations in various codons of Pfmdr1 and Pfcrt will be accessed via RFLP-PCR. Global epigenome signatures associated with the histone modification will be investigated via chromatin immunoprecipitation (ChIP) and DNA methylation by ELISA at these particular *loci*. Comparisons will be made with the controls which consist of the unexposed *P. falciparum* strains. This investigation aims at providing further information into the molecular evolutionary mechanisms of CQ anti-malarial drug resistance.

## 356

### POPULATION GENETIC STRUCTURE, AT A SPATIAL AND TEMPORAL LEVEL, OF *PLASMODIUM VIVAX* IN THE PERUVIAN AMAZON BY GENOTYPING WITH THE QIAXCEL ADVANCED SYSTEM

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Iquitos, the most populated and biggest city in the Peruvian Amazon, is well known for being a hypoendemic region for *Plasmodium vivax*. Consequently, there is little information about the transmission dynamics of *P. vivax* in this region. Therefore, the purpose of this study was to determine the genetic diversity, at a spatial and temporal level, of *P. vivax* in the Peruvian Amazon by genotyping with 15 DNA microsatellites with the QIAxcel Advanced System from QIAGEN. 270 *P. vivax* infected blood samples, which were collected through passive surveillance between the communities of Santo Tomás, San José de Lupuna, and Padrecocha in the city of Iquitos, were genotyped with 15 DNA microsatellites with the QIAxcel Advanced system. The analysis of allelic variation of the monoclonal isolates with complete profiles showed that out of the 15 DNA microsatellites, MS16, MS12, MS8, MS9, and Pv6635 were the most polymorphic ones as defined by their Hunter-Gaston Discrimination Index (HGDI). This makes them the most indicative ones for determining population genetic structure of *P. vivax* in the Peruvian Amazon. The analysis of the complete profiles demonstrated that the vast majority of samples in each of the three sites had unique profiles. In Padrecocha, 60 out of 63 samples had different profiles, in San José de Lupuna, 57 out of 60 had different profiles, and in Santo Tomás 36 out of 39 samples had different profiles. The analysis by UPGMA (Unweighted Pair Group Method with Arithmetic Mean) showed that out of the 162 isolates with complete profiles, there were 9 different groups; each of these could have had descended from a common ancestor. An analysis with the BURST algorithm (Based Upon Related Sequence Types) presented a total of 24 groups that had 13 out of 15 identical *loci*. 75 isolates were considered singletons. The 270 *P. vivax* isolates found in the Peruvian Amazon showed to be genetically diverse, both at a spatial and temporal level, since the analysis by profile determined unique profiles for the vast majority of the isolates. The BURST analysis supports this idea giving 75 singletons out of the 162 isolates with complete profiles.

## 357

### POLYMORPHISM -1447A>G IN CXCL10 GENE PROMOTER SEQUENCE IS ASSOCIATED WITH INCREASED EXPRESSION LEVEL OF CXCL10 AND CEREBRAL MALARIA PATHOGENESIS

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Cerebral malaria (CM) is a neurological complication of *Plasmodium falciparum* infection and a major cause of mortality in children under 5 years of age. Several host and parasite genetic factors have been implicated in CM pathogenesis. The risk factors for CM and the wide variation in clinical manifestations of malaria are poorly understood. Human genetic variation has been shown to influence susceptibility to malaria, progression to CM and death. Recent studies have shown CXCL10, an angiostatic and pro-apoptotic chemokine, to be a strong predictor of both human CM and experimental CM. Increased plasma and cerebrospinal levels of CXCL10 was tightly associated with fatal CM in humans in India and Ghana. Furthermore, it has been demonstrated that increased CXCL10 production in cerebral malaria patients is responsible for inducing apoptosis in brain vascular endothelial and glia cells thereby causing blood-brain barrier dysfunction and damage. In the present study, we hypothesized that in a subset of malaria patients, CM is genetically linked to variation in plasma CXCL10 expression. We wanted to determine whether published polymorphisms in the CXCL10 gene promoter region played a role in the clinical status of malaria patients and address the genetic basis of CXCL10 expression during malaria infection. Two known SNPs in the CXCL10 promoter (-1447A>G and -135G>A) were selected on the basis of their association with CNS disorders and genotyping was performed in 66 CM and 69 non-CM patients using PCR-restriction fragment length polymorphism assay. We found that the -1447A>G polymorphism was significantly associated with susceptibility to CM. Individuals bearing at least one G allele of the -1447A>G polymorphism were susceptible to CM (OR = 2.60, 95% CI = 1.51 - 5.85, p = 0.021). Moreover, individuals with the A/G genotype (-1447A>G polymorphism) had significantly higher serum CXCL10 levels than the AA genotype. The A to G substitution in the -1447A>G polymorphism resulted in the loss of binding site for TATA-binding protein and interferon regulatory factor 4. However, we did not find any association between the -135G>A

polymorphism and CM. Polymorphisms in the CXCL10 gene promoter sequence was associated with high CXCL10 production, which plays a role in severity of CM. These results suggest that the -1447A>G polymorphism in the CXCL10 gene promoter could be partly responsible for the genetic variation underlying susceptibility to CM.

## 358

### FOLATE PATHWAY POSSIBLY ASSOCIATED WITH *PLASMODIUM VIVAX* RELAPSE IN THE PERUVIAN AMAZON BASIN AFTER PRIMAQUINE TREATMENT

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Primaquine (PQ) is used to prevent *Plasmodium vivax* (PV) relapses by eliminating hypnozoites. Two hypothetical mechanisms of action of PQ are thought to be oxidative stress and pyrimidine synthesis inhibition. PV relapses after PQ therapy are suspected to involve complex host and parasite factors, but due to the lack of PV culture assays there is limited empirical evidence. Therefore we studied the association between relapses and mutations in 3 genes encoding folate pathway enzymes, which are precursors of pyrimidine synthesis pathway, and a transporter protein that could be involved in the detoxification process. We studied parasite genetic factors and clinical-epidemiological data associated with relapse, conducting a case-control study within a clinical trial that investigated the efficacy of 3 different doses of PQ to prevent relapses. This study was conducted from 2006 to 2008 in 3 communities in the Peruvian Amazon Basin: Padrecocha, San Juan and Santa Clara. The main result showed that the two study arms with total doses of 210 mg had lower 6-month relapse rates than the arm with total dose of 150 mg. We included 47 individuals who had a PV homologous relapse (relapse cases). The 401 individuals who did not experience a relapse were included as controls for clinical-epidemiological factors (non-relapse controls). DNA sequencing was performed to identify mutations in the *Pvdhfr*, *Pvdhps* and *Pvmdr1* genes in all cases and a random sample of control subjects (n=57). Subjects with weight>58 Kg (HR 2.71, *p=0.012*) and living Padrecocha and San Juan (HR 3.37 *p=0.033* and HR 2.89 *p=0.047* respectively) had increased relapse risk, after adjusting by treatment arm and age. The A383G mutation in the *Pvdhps* gene was associated with more frequent relapses (38% vs 19%, *p=0.032)*. Also, the triple mutant *Pvdhfr* genotype F57/S58R/Y69(TAT>TAC)/S117N was more frequent in relapses than controls (62% vs 38%, *p=0.033)*. *Pvmdr1* was not associated with relapses. The association with *Pvdhfr* remained marginally significant after adjusting by community and weight, despite the small sample size.These findings suggest that mechanisms in folate pathway of the parasite could be involved in relapses after PQ treatment, possibly as a pyrimidine synthesis modulator. More studies are needed to validate these results.

## 359

### CYCLIN-DEPENDENT KINASE PFMRK PLAYS AN IMPORTANT ROLE IN G1/S PHASE TRANSITION IN *PLASMODIUM FALCIPARUM* CELL CYCLE

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Clinical symptoms of malaria result from the rapid growth and cyclic multiplication during erythrocytic schizogony. This process is controlled by the parasite cell cycle regulatory mechanism. A better understanding of this parasite cell cycle regulatory mechanism may lead to means to interrupt parasite growth and replication; and development of novel antimalarial drugs. Cyclin dependent protein kinases (CDKs) are major regulators for growth and proliferation of mammalian cells. Pfmrk, a sequence homologue of human CDK7 is suggested to play an important role in parasite cell cycle regulation. We investigated the role of Pfmrk in both cell cycle regulation and DNA replication in the plasmodial cell cycle using transgenic parasites that over-express functional Pfmrk (HPG), non-functional Pfmrk (HKG) and control (empty vector control). We observed that HPG has accelerated the transition of trophozoites to schizonts while this transition was delayed in HKG. This accelerated transition in HPG was blocked by WR636639, a second-generation chalcone that selectively inhibits Pfmrk, when added to ring-stage parasites. In contrast, when WR636639 was added to trophozoite-stage parasites, the transition from schizonts to rings was delayed. A similar inhibitory effect was also observed in the HKG line.These findings suggest that Pfmrk plays an important role in the transition of G1/S phase, parasite growth and development.

## 360

### MEASURING ERYTHROCYTE SURFACE-ANCHORED PFEMP1 LEVELS AMONG PROGENY FROM A 3D7 X HB3 *PLASMODIUM FALCIPARUM* GENETIC CROSS

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*Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) is the main parasite virulence factor of *P. falciparum* due to its central role in mediating the cytoadherence of infected red blood cells (iRBCs) to the host microvascular endothelium during parasite infection. Directly targeting PfEMP1 as a therapeutic strategy is greatly limited due to the protein's hypervariable nature, which gives rise to approximately 60 different variants. Interfering with the trafficking of PfEMP1 to the iRBC surface is a more attractive therapeutic approach, as hypervariability becomes inconsequential and previous studies have demonstrated that reduced surface PfEMP1 levels significantly weaken cytoadherence, which likely lessens the severity of malaria symptoms. Interestingly, the *in vitro* culture-adapted parasite line 3D7 is inherently defective in exporting PfEMP1 to the iRBC surface. Presuming that PfEMP1 export from the parasite to the iRBC surface is controlled at the genetic level, we hypothesized that 3D7 harbors one or more genetic determinants of impaired PfEMP1 trafficking. To test this, we examined the surface PfEMP1 levels of 17 progeny clones obtained from a genetic cross between 3D7 and the 'trafficking-competent' parasite line HB3. This was accomplished using both Western blotting and a two-color, triple-layer flow cytometry assay with plasma from malaria-immune Malian adults. We found that 3D7 displays 75% less PfEMP1 on the iRBC surface than HB3. Progeny phenotypes normalized to

HB3 range from 37% more to 88% less surface PfEMP1 levels. Using these phenotypes in QTL analysis, we identified significant *loci* on chromosomes 12 and 14 that each explain 50% of the phenotype variance. The role of candidate genes in the trafficking of PfEMP1 to the iRBC surface will be confirmed in allele-exchange experiments, where the defect is rescued in 3D7 and introduced in HB3. The results of this study may strengthen our understanding of malaria pathogenesis and provide new targets for much needed therapeutics.

## 361

### HAPLOTYPIC VARIATIONS IN HSP70 ARE ASSOCIATED WITH ALTERED EXPRESSION OF HSP70, INFLAMMATORY MEDIATORS, AND SUSCEPTIBILITY TO SEVERE MALARIAL ANEMIA

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The present study was designed to validate a genome-wide association study (GWAS) and transcriptome data generated using 'polarized' samples from children infected with *Plasmodium falciparum* malaria (n=48; 3-36 mos.). Although these experiments resulted in the identification of several important genes, here we report the association between markers in the heat shock proteins (Hsp) 70 encoding genes and severe malarial anemia [SMA, hemoglobin level <5.0 g/dL]. Hsp70 is a major stress-inducible protein that acts as a chaperone, immunomodulator, and mediator of antigenic peptide delivery and presentation. The HSP70 gene family is comprised of two nearly identical inducible HSPA1A and HSPA1B genes. Variation in these genes has been implicated in the pathogenesis of several diseases, however, a role in malaria has not been reported. As such, we validated the markers identified in the GWAS [i.e., HSPA1A (-217C/G; -5457A/C; -5893G/A) and HSPA1B (-4439G/A and -8133T/C)] and examined their association with SMA in parasitemic children (n=854). Binary regression analyses controlling for confounders revealed that carriage of the CAGGT, CAAGT, and CAAAT (-C217G/-A5457C/-G5893A/-G4439A/-T8133C) haplotypes were associated with reduced risk of developing SMA [(odds ratio (OR), 0.50; 95% CI, 0.25-0.96; P=0.034) (OR, 0.27; 95% CI, 0.10-0.68; P=0.006) and (OR, 0.36; 95% CI, 0.13-0.95; P=0.041)]. Additional analyses of examining malaria-associated inflammatory mediators demonstrated that non-carriers of the CAAGT haplotype had elevated levels circulating IL-1β,P=0.034), IL-6 (P=0.031), and TNF-α (P=0.008).Gene expression analyses revealed reduced levels of HSP70 transcripts in the SMA group (P<0.001) and elevated HSP70 levels in carriers of the CAAGT and CAAAT (P=0.017 and P=0.015) haplotypes. Furthermore, measurement of circulating Hsp70 revealed elevated levels in the SMA group (P=0.062). Taken together, variation in the promoters of HSP70 increases susceptibility to SMA and functionally alters HSP70 gene expression and inflammatory mediator' production.

## 362

### *IN VITRO* HUMAN CELL FREE EXPRESSION SYSTEM FOR EXPRESSION OF MALARIAL PROTEINS

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Malaria still remains a global concern with no routine vaccines available. Additional vaccine and drug candidate molecules need to be investigated. From previous *Plasmodium yoelii* rhoptry proteome studies, the genes PY01759, PY00763 and a *P. falciparum* PFA0680c were selected. Data from the *Plasmodium* database, PlasmoDB indicates that PY01759 and PY00763 encode proteins of molecular weights 290 kDa and 27 kDa respectively, and are expressed in sporozoite and all erythrocytic stages of *Plasmodium*. In contrast, PF3D7_1361800 the *P. falciparum* orthologue of PY01759 is expressed only in erythrocytic schizonts. Both the orthologs contain Armadillo motifs, which have been shown to play a major role in rhoptry membrane attachment. PFA0680c proteins of molecular weight 25 kDa and the PF3D7_0925900 *falciparum* ortholog of PY00763, encoding a protein of molecular weight 27 kDa are expressed in ring and early trophozoite of the erythrocyte stage. Additional sequence analysis revealed that PY01759 protein is conserved among other apicomplexans. While PY00763 and its *P. falciparum* ortholog PF3D7_0925900 are conserved in *Plasmodium sp.*, PFA0680c is *P. falciparum* specific. In this study, in silico analysis and expression of genes PY00763, PY01759 and PFA0680c was carried out using a new HeLa cell based *in vitro* human cell free expression system. Proteins from the three genes were successfully cloned into plasmid pT7CFE-6his, expressed and purified using Ni-chelating resins. Expressed proteins were identified using rhoptry specific antibodies. The human cell free expression system offers an alternate approach to wheat germ and rabbit reticulocyte lysate cell free expression systems and has the ability to express proteins in three hours.

## 363

### MALARIA PARASITEMIA IN INFANTS RECEIVING IMMUNIZATION IN THE BUEA HEALTH DISTRICT, CAMEROON

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Malaria remains a major cause of mortality and morbidity in infants in sub-Saharan Africa. The intermittent preventive treatment of malaria in infancy (IPTi) has been shown in some settings to be effective in reducing the burden of malaria. However the timing of IPTi in any setting depends on the prevalence and intensity of parasitaemia in infancy. IPTi has not been implemented in Cameroon. However, because contact with health facilities during immunisations represents an opportunity for IPTi, this study sought to determine malaria parasitaemia in infants during immunisation visits in the Buea Health District. A cross-sectional study was conducted in a sample of 220 consenting mother-infant pairs as they came for either the second (DPT2) or third (DPT3) dose of immunisation against Diphteria, Pertusis and Tetanus, or for measles immunisation; administered respectively at 10 weeks, 14 weeks and 9 months of age. A standardised questionnaire was administered to the mothers, after which blood smears collected from the infants were examined for malaria parasitaemia using standard methods. Malaria parasitaemia prevalence at DPT2, DPT3 and measles immunisations were respectively 1.5%, 16.7% and 9.6% (p =0.01). The respective malaria parasite densities were 3.0±25 parasites/µl at DPT2, 16±62 parasites/µl at DPT3 and 3±21 parasites/µl at measles immunisation (p=0.35). No clinical or socio-demographic factors were found to be significantly associated with the presence of parasitaemia. Malaria parasitaemia prevalence was low at DPT2 but relatively high at and after DPT3 suggesting that if IPTi were to be considered in this setting it ought not to start before DPT3. These findings however need to be confirmed in a larger prospective study incorporating an assessment of cost-effectiveness.

## 364

### USING AN ODOR-BAITED DEVICE THAT MIMICS HUMANS TO EXPLORE TECHNICAL OPTIONS AND CHALLENGES FOR ATTRACTING AND KILLING OUTDOOR-BITING MALARIA VECTORS

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Mosquitoes that bite people outdoors can sustain malaria transmission even where effective indoor interventions such as bed nets or indoor residual spraying are already widely used. Outdoor tools may therefore complement current indoor measures and improve control. We developed and evaluated a prototype mosquito control device, the 'Mosquito Landing Box' (MLB), which is baited with human odours and treated with

mosquitocidal agents. We conducted field experiments in Tanzania to assess if wild host-seeking mosquitoes 1) visited the MLBs, 2) stayed long or left shortly after arrival at the device, 3) visited the devices at times when humans were also outdoors, and 4) could be killed by contaminants applied on the devices. Odors suctioned from volunteer-occupied tents were also evaluated as potential low-cost bait, by comparing baited and unbaited MLBs. There were significantly more *Anopheles arabiensis*, An. *funestus*, *Culex* and Mansonia mosquitoes visiting baited MLB than unbaited controls (P≤0.028). Increasing sampling frequency from every 120 min to 60 and 30 min led to an increase in vector catches of up to 3.6 fold (P≤0.002), indicating that many mosquitoes visited the device but left shortly afterwards. Outdoor host-seeking activity of malaria vectors peaked between 7:30 and 10:30pm, and between 4:30 and 6:00am, matching durations when locals were also outdoors. Maximum mortality of mosquitoes visiting MLBs sprayed or painted with formulations of candidate mosquitocidal agent (pirimiphos-methyl) was 51%. Odours from volunteer occupied tents attracted significantly more mosquitoes to MLBs than controls (P<0.001). Odor-baited devices such as the MLBs clearly have potential against outdoor-biting mosquitoes in communities where LLINs are used. Natural human odors suctioned from occupied dwellings could constitute affordable sources of attractants to supplement odour baits for the devices. To curb risk of physiological insecticide resistance the killing agents used should be of different modes of action (other than pyrethroids as used on LLINs).

## 365

### FAUNA INVENTORY AND ASSESSMENT OF *CULICIDAE* NUISANCE IN POST-CONFLICT URBAN AREA: THE CASE OF BOUAKÉ, CÔTE D'IVOIRE

Long Lasting Insecticidal bed Nets **(**LLINs) are one of the most effective and feasible option to control the malaria vectors in endemic zones. Among them, PermaNet®2.0 has proven its efficacy in the field conditions with a remanence of 3 years and a resistance at 20 washes. Data is lacking on assessment of the LLINs efficacy when intensively used in hardship conditions such as in the forest. The aim of the current study was to evaluate in the laboratory conditions the physical status and bio-efficacy of PermaNet®2.0 after 18 months of use in an isolated forest camp in Pokola, Congo. This study was conducted at the CSRS in Côte d'Ivoire on 51 used bed nets originated from Congo. The evaluation included the physical status of the bed-nets that were collected to enumerate the holes and their position, repairs, seam failure, nets size and the assessment of the bio-efficacy according to WHO criteria. The Knock Down (KD) and the mortality rates after 3 minutes exposure of susceptible strains *An. gambiae* Kisumu to mosquito nets were determined respectively after 1 hour and 24 hours post-exposure. Among 51 bed-nets collected, only, 5 nets (9,8%) were in good condition (no hole, white colour). A total of 990 holes were observed corresponding to an average of 19 holes per net. The number of holes was much higher on the big face than on the roof. Furthermore, the majority of holes were found on the border and the lower part of bed-nets, contact points with the bed. There were only 55 repairs counted corresponding to 1,07 repair per net. All the nets were too dirty becoming grey (74,5%) and lost their original color. An increase in nets size was observed with a difference varying between 9 and 26 cm comparing to the original size. Bioassay results indicated a low mortality rate on different sides of the nets to the standard set by WHO (80-100%). However, high knock down rates was found on the roof (98%), 95,3% on the length and 94.0% on the width. The knockdown values complied with WHO criteria (knockdown ≥ 95%). In conclusion, the current study showed that even when PermaNet®2.0 bed nets are torned and are dirty, they still conserve their efficacy even after 18 months in the field.

## 366

### TRANSCRIPTOME PROFILING OF THE PYRETHROID RESISTANT AND SUSCEPTIBLE MOSQUITOES IN THE ASIAN MALARIA VECTOR, *ANOPHELES SINENSIS*

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*Anopheles sinensis* is one of the major vectors of *Plasmodium vivax* malaria in Asia.Very limited genomic information regarding *An. sinensis* is currently available in public databases, comparing to other malaria vectors in Africa, e.g., *An. sinensis*. RNA-seq provides a fantastic approach to advance the genetics and genomics of *An. sinensis*. To generate novel genomic sequence information for this species and discover transcripts involved in insecticide resistance, we sequenced the transcriptomes of lab colony of *An. sinensis*, as well as those from field pyrethroid resistant and susceptible mosquitoes in China. 454 GS-FLX transcriptome sequencing yielded a total of 624,559 reads (average length of 290bp) using the pool of lab *An. sinensis* colony and field mosquitoes. The de novo assembly generated 33,411 contigs with average length of 493bp. A total of 8057 final ESTs were generated with the Geneontoly (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) annotation. Moreover, 2326 unigens were identified to be the differentially expressed unigenes (DEGs) based on the Reads Per Kilobase per Million mapped reads (RPKM) in field deltmathrin resistant (FR) and susceptible (FS) mosquitoes. There are 261 pathways in DEGs that mapped to the KEGG database, with the predominant unigenes involved in 'Metabolic pathway'. In addition to the P450 Monooxygenases and other metabolic detoxification genes, the distribution of the midgut bacterial and immune genes were also contributed to the DEGs. Furthermore, 2,489 microsatellites were identified and a total of 15,496 sites were estimated to contain Single Nucleotide Polymorphisms (SNPs) and the SNPs of open read frame in 15 contigs were analyzed.The assembled and annotated transcriptome databases provide a significant valuable genomic resource for further study on the important Asian vector, the differential unigenes identified in this study put forward abundant genetic information for further understanding of the molecular mechanism of insecticide resistance. The identified microsatellite and SNP markers will prove useful for extending our current knowledge of the genome organization, for whole genome association studies, and for carrying out comparative genomic analyses within the *Anopheles* mosquito species.

## 367

### A STUDY OF THE BITING PATTERN WITHIN *ANOPHELES GAMBIAE SENSU LATO*

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The recent evidence indicating a modification in the biting pattern of vector populations could pose a challenge to the increased use of Insecticide treated bed nets as a malaria vector control strategy. This modification includes adaptation to biting humans when they are out of bed that is early evening and dawn together with increased outdoor biting. This modification might not necessarily be as a result of an adaptive change in vector behaviour but due to selective pressure against mosquitoes biting when bed nets are in use. This study therefore determined if discrete vector populations with specific biting times exist within *Anopheles gambiae* populations. *An. gambiae* sampled from 6pm to 6am in the field were pooled into four categories based on their time of biting; 6pm-9pm, 9pm-12am, 12am-3am and 3am-6am. Generations were raised on which feeding experiment and selection were performed.

Non anophelines were separated from anophelines and PCR run on *A. gambiae s.l.* for species identification and molecular forms. *Anopheles* sampled comprised of *An. sinensis* (99.3%), *An. funestus* (0.5%) and *An. pharoensis* (0.1%) [n=744]. Different numbers of F1 and F2 progeny were obtained and used for feeding experiments for the different time groups. The percentages for the F1 groups that fed at same time periods as parents in the field (compared with feeding at other time points pooled together) were 90.5%(P=0.013) for 9pm-12am, 66.3%(P=0.029) for 12-3am and 62.3%(P=<0.0001) for 3-6am, whilst F2 had 100%(P=0.228) for 9-12am and 91.2%(P=0.037) for 12-3am. However, irrespective of the period F1 and F2 from different collection times were exposed to feed, a high proportion was found to take a blood meal. PCR run on 134 *An. gambiae s.l.* showed 100% *An gambiae s.s.* Further molecular forms which were determined using restriction enzymes gave 94.8% S forms and 5.2% M forms. Vector behaviours such as host preference, biting pattern and resting have been have been linked to genetic influence but results obtained for F1 and F2 generations showed otherwise probably due to extrinsic factors.

## 368

### BEYOND BUZZING: MOSQUITO WATCHING STIMULATES MALARIA BEDNET USE

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Malaria remains a leading morbidity and mortality cause in Africa. Insecticide treated nets (ITNs) are effective for malaria control. Many organizations have distributed free or highly subsidized ITNs in endemic areas. Nevertheless, some recipients do not use ITNs because of social, environmental or cultural factors. Health education may improve ITN use among people owning but not using an ITN. Here, we hypothesized that watching freshly collected-alive and buzzing-mosquitoes in a household could increase ITN use. To test the hypothesis, we conducted randomized educational intervention in Zomba district, Malawi. Our study site consisted of 285 households and 1199 inhabitants when we began the study. After a series of surveys including bednet distributions, we finally determined that 36 households, which had contained at least one member owing but not using an ITN, were eligible for educational intervention. These 36 households were randomly divided into three educational groups: control (n = 12 households (HHs), 33 people (PP)), education with leaflets (n = 11 HHs, 32 PP), and education with leaflets and fresh mosquitoes (n = 13 HHs, 31 PP). The outcomes were measured by individual ITN uses and household heads' knowledge. The results showed that people who watched freshly collected mosquitoes were about 10 times more likely to use ITNs than those who saw only an educational leaflet or a control group. However, knowledge differences among the groups were not observed. Our results suggest that vector presence realization by direct observation can encourage ITN use and may potentially improve effective ITN coverage for malaria control and elimination.

## 369

### HOW CAN HEALTH AND DEMOGRAPHIC SURVEILLANCE SYSTEM (HDSS) PLATFORM CONTRIBUTE TO MEASURE INSECTICIDE TREATED NETS (ITNS) EFFECTIVENESS IN WEST AFRICAN COUNTRY: EXPERIENCE OF NOUNA HDSS IN RURAL BURKINA FASO?

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Malaria remains the global cause of morbidity and mortality, with most of the burden being in sub-Saharan Africa. Insecticide Treated Nets (ITNs); one of the most effective strategies of Roll Back Malaria is currently rolled out on a large scale. However no much is known about the coverage target and the effectiveness of such a strategy. The aims of the study were to assess the coverage and use of ITNs among pregnant women and under 5 years and its effects on malaria morbidity and mortality in Burkina Faso. A cross-sectional household survey was conducted in the Nouna Health and Demographic Site (NHDSS) in 2011 after the large national free distribution of ITNs. Data were collected from a total of 1050 households, which included 1202 pregnant women and 1114 children using a three-stage cluster sampling procedure in 59 villages of the NHDSS. Overall 97 % of households revealed a possession of at least one ITN in 2011 compared to 89% in 2009. In 2011, 68.6% of children have slept under ITN the last night compared to 25% in 2009. It was 69 % for pregnant women compared to 28% in 2009. The prevalence of clinical malaria among less than five years increased from 20% in 2009 to 21% in 2011. Meanwhile the malaria mortality decreased from 4% in 2009 to 2% in 2011 showing an ITN protective effect (OR=0.66; 95%CI: 0.54-0.80; P<0.05) in target population. In conclusion, although significant progress have been made to improve access of population to ITNs resulting in slight decrease of malaria mortality in NHDSS, still much remains to do for achieving the universal coverage of ITNs needed to attain the MDGs goals . Emphasis should be put on pregnant women and children under five years to ensure their equitable access to malaria prevention materials.

## 370

### ASSOCIATION BETWEEN HOUSE STRUCTURE AND THE INCIDENCE OF MALARIA IN AFRICAN CHILDREN

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Insecticide treated bednets (ITNs) are the most widely adopted intervention for the prevention of malaria in African children. Improved house structure may lower the density of mosquito vectors, offering an additional means of reducing the risk of malaria. This study explored the relationship between materials used in house construction and the incidence of malaria in a cohort of children followed prospectively as part of a randomized clinical trial of antimalarial chemoprevention. A total of 600 children aged 4-5 months living in different houses were enrolled using convenience sampling in Tororo, a rural area with perennial high transmission intensity. Children received an ITN at enrollment and were followed for all their health care needs 7d/wk. Children were randomized to 1 of 4 chemoprevention arms: HIV unexposed infants were randomized at 6 months of age and HIV exposed children at aproximately 6 weeks after cessation of breast-feeding (median age 10 months). Approximately 1 year after the start of the study a survey was performed at each child's house detailing the materials used in house construction. Associations between house structure and the incidence of laboratory confirmed malaria between 6-24 months of age by passive surveillance were estimated using a negative binomial regression model, with measures of association expressed as the protective efficacy (PE=1-incidence rate ratio). The final analyses include 515 children. The prevalence of houses with non-earth floors, non-thatched roofs, and non-mud walls was 14.6%, 43.1%, and 17.5%, respectively. After controlling for chemoprevention, the incidence of malaria was 64% lower in those with a non-earth floor (95% CI, 53-72%, p<0.001), 27% lower with a non-thatched roof (95% CI, 14-39%, p<0.001), and 53% lower with non-mud walls (95% CI, 42-63%, p<0.001). Houses constructed with all non-natural materials were associated with a 70% lower incidence of malaria (95% CI, 60-77%, p<0.001).Most houses in this rural area were constructed with basic materials. Compared to these, houses constructed with materials other than earth, mud, and thatch were associated with a lower incidence of malaria.

## 371

### USE OF INSECTICIDE TREATED NETS AS A CONTROL STRATEGY FOR MALARIA IN WESTERN KENYA

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Globally, 500 million cases of malaria occur annually with an estimated 2.7 million deaths of which 80-90 % are in Sub-Saharan Africa. By 2004, 107 countries were affected and 2b people at risk. In Kenya 30-50% of the out patients are due to malaria, 19% admissions and 42,000 deaths annually. Malaria is an increasing problem among expectant mothers and children under ten and is a major public health problem among the communities within the sugarcane Belt in Kenya. While malaria burden is ever on an increasing, vector control remains a neglected option for malaria control. Intermittent preventive treatment (IPT) with SP drugs has been used in Sub Saharan Africa and is currently the recommended policy in Kenya. However, increasing resistance to SP limits its use for malaria control. Insecticide Treated Nets (ITNs) have been shown to reduce the number of mosquito bites and in turn reduce all cause morbidity and mortality among expectant mothers and children. The objective of the study was to assess the use of ITNs and determine prevalence of malaria and anaemia in expectant mothers attending ANC during high transmission season. To demonstrate the effectiveness of ITNs in community set-ups, 387 expectant mothers attending ANC in Western Kenya located in a malaria endemic area with two high transmission seasons were recruited in. Presence of malaria parasitaemia and hemoglobin concentration, the presence/ absence of fever were determined by measuring temperature of the recruited patients. Of the 387 expectant women recruited, 190(49.1%) of them owned nets, either conventional or long lasting ones. Prevalence of malaria parasitaemia was 28.9%, of which 19.8% (77/387) had malaria (≥ 800 mps/ml of blood). 32.80%, 21.64% and 9.09% of primigravidae, secondigravitae and multigravitae respectively had moderate anaemia (5.0g/dl>7.00g/dl). Parity and net use were significantly associated with malaria status. Occupation was not significantly associated with net ownership and use, pointing to the possible success of awareness campaigns and favorable price affordable to majority. Marital status and education were found to be strong predictors of net ownership and use. It was concluded that net use reduced malaria prevalence during pregnancy.

## 372

### A HIGH-THROUGHPUT LUCIFERASE-BASED ASSAY IDENTIFIES INHIBITORS OF DEVELOPING *PLASMODIUM FALCIPARUM* GAMETOCYTES

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The design of new therapeutic combinations against *Plasmodium falciparum* malaria requires novel drug candidates able to interrupt the disease transmission to the mosquito vector. *P. falciparum* gametocytes, which develop in the human host over a 10 days period, represent the most accessible target stage for such drugs. Considerable effort is currently being taken to identify compounds with late stage gametocytocidal activity, however investigations on the drug sensitivity profile of developing gametocytes, as well as screening methods for early gametocytogenesis inhibitors remain scarce. We developed and optimized a luciferase-based high-throughput screening assay on tightly synchronous early stage *P. falciparum* gametocytes, using a recombinant Pfs16-driven GFP-luciferase NF54 parasite line. The 384-well format assay encompassed gametocyte development from stage I to IIb/III over 72 hours, and was validated by small-scale screening of a collection of 400 compounds with known antimalarial (asexual stage) activity. The assay provided excellent performance with average %CV ≤ 5%, signal to noise ratio above 30 and Z' near 0.8. Only 135 compounds from the collection (1/3 of the total) inhibited early gametocytes at least 50% at 5 µM. The 5 most active hits showed IC50 values around 200 nM. No correlation was found between the gametocytocidal IC50 values of the screening hits and their potency against *P. falciparum* 3D7 asexual stages. Our HTS assay proved reproducible and suitable for the screening of large compound libraries on developing gametocytes. Within the transmission blocking drug discovery strategy, our findings highlight the necessity of screening efforts directed specifically against early gametocytogenesis, and warrant the inclusion of early stage gametocytocidal activity in the desired Target Candidate Profile for antimalarial drug development.

## 373

### USING MALE MOSQUITOES TO CROSS-CONTAMINATE CON-SPECIFIC FEMALES WITH LETHAL DOSES OF PYRIPROXIFEN DURING COPULATION: AN OPTION FOR FUTURE MALARIA CONTROL

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Existing prevention methods such as bednets have significantly reduced malaria cases, but there is consensus that complementary tools are necessary to address ongoing challenges. Studies have shown that *Aedes egypti* mosquitoes, which transmit dengue virus, can pick up lethal doses of pyriproxifen (PPF) during resting and transfer these to their larval breeding sites during oviposition, thus effectively stopping further aquatic mosquito development. Furthermore, it has been demonstrated that males of *Aedes* can contaminate their females during mating. Here, we assessed the possibility of using male *Anopheles arabiensis* to cross-contaminate counterpart females with PPF during copulation, and whether this could reduce fecundity of the females and viability of the resulting eggs. A cup of *An. arabiensis* pupae were put inside a netting cage dusted with 10% PPF, after which 50 emerged males were aspirated into a clean cage in which there were 100 uncontaminated con-specific females of the same age. The mosquitoes were left for 6 days to mate, and then the females were blood fed for 3 consecutive days and provided with egg-laying pots. Relative to a control cage of clean males and females, we assessed number of eggs laid and proportion of the eggs that hatched. Lastly, by dipping the adult males and females into cups with clean 3rd-instar larvae, then assessing larval development to pupation and emergence, we verified whether the males had picked and carried PPF, and determined efficacy of PPF particles potentially picked by the females during copulation. The number of eggs from control cages was significantly higher than from the PPF-dusted cage (P=0.011); the mean number of eggs laid was 12.22 [9.52-14.92] in control and 5.90 [5.35-6.44] in treatment. 37% of the eggs from control hatched compared to 30% in the treatment. Where males from PPF-dusted cage were dipped into larval cups,85% emergence inhibition was observed compared to 6% in control. Similarly, where females mated with the contaminated males were dipped,96% emergence inhibition was seen compared to 1% in control. Pyriproxyfen contaminated males can successful contaminate con-specific females during mating and significantly reduce their fecundity and hatchability rate. With further research, male mosquitoes could potentially be used as a means to disseminate pyriproxyfen in an effort to eliminate malaria though vector population control.

## 374

### DEVELOPMENT OF A GRAVID TRAP FOR COLLECTING MALARIA VECTOR *ANOPHELES GAMBIAE S.L.*

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Effective malaria vector control targeting indoor host-seeking mosquitoes has resulted in a reduction in the number entering houses in many

areas of sub-Saharan Africa, with the proportion of vectors outdoors becoming more important in the transmission of this disease. This study was intended to develop a gravid trap for the out-door collection of the malaria vector *Anopheles gambiae s.l.* based on evaluation and modification of commercially available gravid traps used for culicine collections. Experiments were implemented in an 80 $m^2$ semi-field system where 200 gravid *An gambiae s.s.* were released each night and replicated for 12 nights. The catching efficacy of the Box, CDC and Frommer updraft gravid traps was compared. Later the Box gravid trap was tested to determine if the presence of the trap over water and the trap's sound affected catch size. Mosquitoes approaching the treatment were evaluated using electrocuting nets or detergents added to the water in the trap. Based on the results of these experiments, a new gravid trap that provided an open, unobstructed oviposition site was developed and evaluated. Box and CDC gravid traps collected similar numbers of mosquitoes (Odds ratio (OR) 0.8, 95% confidence interval (CI) 0.6-1.2; $p = 0.284$), whereas the Frommer updraft gravid trap caught 70% fewer mosquitoes than both traps (OR 0.3, 95% CI 0.2-0.5; $p < 0.001$). The number of gravid females approaching the Box trap was significantly reduced when the trap was positioned over a water-filled basin compared to a small pond (OR 0.7 95% CI 0.6 - 0.7; $p < 0.001$). This effect was not due to the sound of the trap. Catch size increased by 60% (OR 1.6, 1.2 - 2.2; $p = 0.001$) with the new trap. In conclusion, gravid *An. gambiae s.s.* females were visually deterred by the presence of the trapping device directly over the oviposition medium. Based on these investigations, an effective suction gravid trap was developed that provides open landing space for egg-laying *Anopheles* mosquitoes.

## 375

### RAPID INCREASE IN MALARIA SERVICES FOR PREGNANT WOMEN IN SOUTH SUDAN

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Administration of two doses of intermittent preventive treatment (IPT) with sulfadoxine/pyramethamin (SP) and consistent use of LLINs reduces pregnancy complications and neonatal deaths from malaria. In 2010, a household survey in South Sudan showed that only 22.7% of pregnant women received two doses of SP. The second phase of the Sudan Health Transformation Project (SHTP II) focused on prevention of malaria in pregnancy. By outreach to pregnant women to increase attendance at ANC services, training of maternal health provider staff in IPT, and provision of medications and commodities with minimal stockouts, the SHTP II aimed to increase the number of pregnant women who received IPT 2. In addition, distribution of LLINs during ANC and under 5 services increased sleeping under an LLIN. During the three years of the SHTP II, IPT2 visits increased 229% and coverage of IPT 2 increased from from 29% to to 53%. ANC 1 increased from a basleine of 53% to 83% of PW, and ANC 4 increased from 23% to 49% of PW. In conclusion, significant increases in utilization of ANC 1 And ANC 4 services improved both IPT 2 and LLIN distribution and use, contributing to overall improvements in maternal and newborn care. Increasing access to malaria in pregnancy prevention efforts by integrating community awarenes, outreach and improved service delivery is possible even in the challenging fragile state of South Sudan.

## 376

### TARGET SITE AND METABOLIC MECHANISMS CAUSE EXTREME INSECTICIDE RESISTANCE AND CROSS-RESISTANCE IN *ANOPHELES GAMBIAE S.S.* FROM WEST AFRICA

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Malaria control depends on mosquito susceptibility to insecticides. With resistance to pyrethroids and DDT now widespread, carbamates are an increasingly important alternative for indoor residual spraying (IRS). Yet mechanisms of resistance to carbamates are poorly understood and critical knowledge of potential cross resistance with other insecticide classes is lacking. We assayed insecticide resistance in *Anopheles gambiae* mosquitoes from southern Côte d'Ivoire and applied synergist, target site assays and whole genome microarrays to investigate resistance mechanisms. Mosquitoes were resistant to insecticides from all four approved classes. Such complete resistance, which includes exceptionally strong phenotypes, presents a major threat to malaria control. The G119S target site resistance mutation was strongly associated with bendiocarb survivorship, but bioassays with PBO, which synergises P450 enzymes, restored significant insecticide efficacy, suggesting a previously unappreciated role of metabolic resistance in carbamate resistance. This observation was confirmed by microarray analyses, which implicated the involvement of multiple P450s in carbamate resistance. The role of the strongest candidate P450, Cyp6M2, which is also linked with pyrethroid and DDT resistance in *An. gambiae* was validated via production of a bendiocarb resistant phenotype in transgenic Drosophila melanogaster. Our results demonstrate strong roles for target site and metabolic mechanisms in producing extreme levels of carbamate resistance in *An. gambiae*, in addition to a concerning potential for cross resistance via overexpression of a specific P450 gene.

## 377

### HOUSE DESIGN MODIFICATIONS FOR "MOSQUITO-FREE HOMES": AN INNOVATIVE, EFFECTIVE AND ENVIRONMENTALLY SOUND ALTERNATIVE TO CHEMICAL USE

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Mosquito-proofing homes have been a fundamental technique for malaria control since early 1900s. Simple non-chemical modifications of a typical rural house design using ventilated ceilings mats can be an effective, acceptable and relatively inexpensive method of reducing entry of indoor mosquito vector densities and consequently decreasing malaria transmission. Ten treatment houses were modified with ventilated ceilings of papyrus mats and insecticide treated netting and tested against ten control houses. To determine densities of mosquitoes resting in homes the pyrethrum spray method was used to simultaneously collect indoor resting malaria vectors in intervention and control houses. Each house was sampled a total of 8 times for 4 months, resulting in a total of 80 sampling efforts for each treatment. Community response was investigated by a questionnaire survey. House modification reduced malaria vectors house entry by 78-86% compared to unmodified houses. Geometric mean density of *An. gambiae s.l.* and An. *funestus* in modified houses were significantly lower ($t158=6.35$, $P<0.0000$ and $t158=2.79$, $P=0.006$, respectively) compared to controls. There was a 84% (OR 0.16, 95% CI 0.07-0.39, $P=0.0000$) and 87% (OR 0.13, 95% CI 0.03-0.5, $P=0.0004$) reduction in the odds of *An. gambiae s.l.* and An. *funestus* presence in modified houses, respectively, compared with unmodified houses. A survey in 270 households indicated that 99% of the respondents had willingness and ready to modify their houses for malaria control. Other reasons

associated with the modification that were cited by respondents to favour the strategy were temperature regulation (50%) and house beautification (33%). Cost was cited as the main challenge where 26% against 68% said was affordable. House modifications involving ventilated ceilings have the potential to reduce human exposure to malaria vectors, and thus parasite infection in malaria endemic regions and likewise reduce the use of chemicals in the environment. Ceilings made from locally available materials are likely to be well accepted.

## 378

### HOW THE MALARIA VECTORS ADAPT TO THE USE OF INSECTICIDE-TREATED NETS BY AFRICAN POPULATIONS?

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Malaria remains one of the main health scourges affecting Africa. The fight against this disease is based on vector controls that rely primarily on indoor residual spraying and the use of long lasting treated bed nets (LLINs). However the effectiveness of these tools now faces the emergence of insecticide resistance and adaptation. This study evaluates the consequences of bed nets use on vectors resistance to insecticides, their feeding behavior in malaria transmission in a rural Senegalese village, Dielmo after deployment of LLINs in July 2008. Adult mosquitoes were collected by human landing catches (HLC) monthly from January 2006 to March 2013 and by pyrethrum spray catch (PSC). Anopheline identification was performed by loupe and sub-species by PCR. The presence of circumsporozoite protein (CSP) of Pl. *falciparum* and the blood meal origin was detected by ELISA, and kdr mutations were investigated by PCR. We demonstrated that, after bed nets implementation, insecticide susceptible mosquitoes (wild kdr genotype) had a reduced lifespan, but they rapidly adapted their feeding behavior, becoming more exophageous and zoophilic, and biting earlier during the night. In the meantime, insecticide-resistant specimens (kdr L1014F genotype) increased in frequency in the population, with an unchanged lifespan and feeding behavior. Better, mosquitoes collected in February-March 2013 between 7pm to 11am by HLC showed that over 65% of anophelines were caught in daylight (07am and 11am). These disturbing results show anopheles adaptative capacity to circumvent strategies aimed at reducing malaria transmission. Due to their extraordinary adaptative skills, *Anopheles* mosquitoes continue to be excellent malaria vectors even after mass deployment of insecticide treated bed nets. Thus, by using nets to protect us, are we not going to provide the *Anopheles* with the entire "arsenal" needed to hit much harder?

## 379

### LABORATORY EFFICACY OF THE PYRROLE INSECTICIDE, CHLORFENAPYR ALONE OR IN COMBINATION WITH PYRETHROID FOR CONTROL OF PYRETHROID SUSCEPTIBLE AND RESISTANT MOSQUITOES

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Pyrethroid resistant mosquitoes are becoming increasingly common in parts of Africa. There is an urgent need to develop alternative insecticides for use in indoor residual spraying (IRS) to supplement the pyrethroids for malaria control. Because there is limited number of new public health insecticides coming onto the market since the event of pyrethroids, certain compounds from the agricultural portfolio of insecticides such as the pyrrole chlorfenapyr have already shown great potential for control of pyrethroid resistant mosquitoes. I furthered on to explore under laboratory conditions the potential of putative dosages of a wetable powder (WP) formulation of chlorfenapyr for IRS against anopheles and the possibility of combining such formulation with the pyrethroid alphacypermethrin for IRS to control both resistant and susceptible mosquitoes, on wooden and cement substrates. Chlorfenapyr WP range 200-500mg/m2 was very effective against anopheles ($P<0.05$) but activity on cement fell short just after 1 month. The combination of chlorfenapyr/alphacypermethrin was also very effective and controlled both resistant and susceptible mosquitoes. Further studies would be needed in experimental huts to estimate the mass killing impact of chlorfenapyr WP alone and its combination with pyrethroid. More advanced technology such as micro encapsulation is needed to enhance the residual life of this useful insecticide on concrete.

## 380

### AN ANALYSIS OF THE COST OF FITNESS OF KDR INSECTICIDE RESISTANCE IN *ANOPHELES GAMBIAE* MALARIA VECTORS

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Previous studies have shown that insects suffer a fitness cost when they try to resist insecticides in their environment. The rationale for this work is to determine if the fitness cost will have a negative impact on wild resistant *Anopheles gambiae s.s.* To achieve this, 100 bloodfed adult female mosquitoes were collected from two villages, Okyereko (resistant) and Dodowa (susceptible) and given optimal conditions to lay eggs which were bred to adulthood and fitness parameters determined. Results obtained indicated that adult survival were 20 and 40 for Okyereko and Dodowa respectively. Analysis from binary logistic regression reveals that average egg laid were higher for kdr negative (178,139) than for kdr positive (100,127) for both sites Okyereko and Dodowa respectively. Similarly, fecundity (139,44) and growth in terms of productivity were both higher for *kdr* - than for *kdr* + however, egg retention was lower for *kdr* - (39,44) than for *kdr* + *An. gambiae* (100,37).Average survival days was also higher for *kdr* - (6,7) than for *kdr*+ (0,3), wheras longevity was (21,16) and (0,10) respectively for *kdr*- and *kdr*+ mosquitoes. A fitness cost was found between *kdr* and longevity as above binary analysis proved longevity of the *An. gambiae* mosquitoes to be significant $P\leq0.05$.This finding of a fitness cost with respect to longevity may prove useful in reducing ability of the malaria vector to transmit malaria parasites.

## 381

### FUNGAL APPLICATION ON NETTING SUBSTRATE FOR MALARIA VECTOR CONTROL IN GHANA

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Recent malaria control strategies, which partly involve the use of fast killing chemical agents to prevent infectious mosquito bites, are being threatened globally by the gradual development of mosquito resistance due to selection pressure. Fungal entomopathogens show.potential as an alternative biological control agent against anophelines. Like conventional insecticides, fungal spores act via contact, typically killing the mosquito in 4-10days, depending on exposure dose, viability, and virulence of the fungal strain. This study aims at determining if the fungus, Beauveria bassiana, can be delivered to mosquitoes on netting materials which could be used in households in Ghana. Anopheline larvae will be collected over a period from three select communities in southern Ghana and raised in the insectary. A minimum of twenty-five (25) each of cotton and polyester bed nets will be treated with spores of B. bassiana suspended in Shellsol solvent by dipping. Three portions of the individual nets will be cut out (30x30cm) at randomly selected positions and stored in aluminum foils for cone bio-assays. The first generation (F1) adults from the larval collection will be used for WHO cone bio-assays on the netting materials cut out

earlier, with controls setup, and observed over a period of sixteen (16) days after exposure. These cut out net pieces will be stored in optimal conditions for the tests to be repeated every week to determine the rate of decay of the spores as well as the longevity of their action. Results are expected to exploit the possibility that bed nets treated by dipping in a suspension of fungal spores dissolved in Shellsol would be a promising alternative for field implementation in Ghana. Biological control with fungus-impregnated netting materials could provide a means to target host-seeking mosquitoes upon house entry, and has potential for use in integrated vector management strategies, in combination with chemical vector control measures, to supplement malaria control in areas with high levels of insecticide resistance.

## 382

### EXPERIENCES OF BENIN REGARDING INDOOR RESIDUAL SPRAYING (IRS) IMPLEMENTATION: PERFORMANCES AND LIMITS

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The National Malaria Control Program (NMCP) has been implementing a vector control strategy based on Indoor Residual Spraying (IRS), using bendiocarb insecticide, in Benin since 2008. As a matter of fact, Benin decided to use IRS especially in order to reinforce the action of the Long Lasting Insecticidal Nets (LLINs). From 2008 to 2010 and 2011 to 2012, IRS was respectively implemented in the Southern Benin, precisely in Oueme department, and in the North, precisely in Atacora department. These 2 trials were carried out at large scale, covering more than 500,000 inhabitants but in a context of high resistance of *Anopheles gambiae* to pyrethroids. And, that is why the IRS strategy adopted was based on the use of a non-pyrethroid, the bendiocarb insecticide. The application dose was 0,4g/m$^2$ of bendiocarb on the walls. Indeed, applications were undertaken by volunteers selected in the community and the coverage rate was more than 85%. To perform any spraying, a manual atomizer with previous pressure HUDSON XPERT type was used by the sprayers who, prior to any application, were protected with a lab coat, gloves, boots and a helmet for their own security. Entomological parameters registered in the control areas were then compared to those of intervention sites. The results obtained were actually encouraging. In southern Benin, the study has shown a drastic decrease in *An. gambiae* biting rate in areas under IRS intervention. Besides, ELISA tests were negative for circumsporozoite (CS) antigen of *Plasmodium falciparum* during the whole period of intervention. Moreover, nobody received infected bites (EIR = 0 from January to July). Similar results were recorded in the North, in Atacora department, with more than 70% malaria transmission reduction. In Benin, bendiocarb insecticide was found to be a good alternative for IRS strategy in areas where *An. gambiae* has developed a high resistance to pyrethroids. However, after 2 years of IRS in the North, bendiocarb resistance in *An. gambiae* due to the use of high quantity of various insecticides by farmers against cotton pests was registered. Therefore, facing the emergence of bendiocarb resistance, the NMCP decided to replace this insecticide by pirimiphos methyl (OP) in 2013. We wonder what will become of IRS in Benin after pirimiphos methyl resistance. Indeed, the resistance has been noted for all types of insecticide.

## 383

### AGE-DEPENDENT SUSCEPTIBILITY OF MOSQUITOES TO IVERMECTIN

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Ivermectin administration to humans can kill mosquitoes and may impact malaria transmission, as reported previously. Several other studies have demonstrated that ivermectin affects *Anopheles gambiae* survivorship, delays re-feeding rates, increases knockdown, delays recovery, reduces fecundity, and multiple blood meals with ivermectin compound mosquito mortality. As reported, susceptibility of mosquito vectors to insecticides has been demonstrated to be age-dependent and upon ingestion of such insecticides, several detoxification mechanisms could be induced more or less efficiently depending on mosquito age. We are investigating the age dependent susceptibility to ivermectin in *Anopheles gambiae* in order to evaluate ivermectin mass drug administration as a new and integrated malaria control tool. Our hypothesis is that ivermectin may induce overexpression of some detoxification genes to eliminate the compound before it reaches its target. and the extent of this induction may be age dependent. We performed blood-feeding experiments with ivermectin on 2, 6 and 14 days post emergence. Preliminary results support the hypothesis of age-dependent toxicity of ivermectin to mosquitoes. Planned experiments will measure the activity of three types of metabolic enzymes involved in xenobiotic detoxification (carboxylesterase, glutathione-S-transferase and P450-monooxygenase) to determine their impact on ivermectin tolerance and on age-dependent susceptibility. We will also perform transcriptomic analysis using RNA-seq to determine the genes induced by ivermectin with special emphasis on detoxifying genes, and to compare the age effect on the expression of these genes. We expect these data will identify potential metabolic resistance mechanisms to ivermectin in malaria vectors. Such data would help to evaluate the strategy of using ivermectin as a systemic drug to control malaria transmission.

## 384

### INVESTIGATION OF DETOXIFICATION GENE PROFILES IN DELTAMETHRIN RESISTANT *AEDES AEGYPTI* POPULATIONS FROM DISTINCT GEOGRAPHICAL ORIGIN

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*Aedes aegypti* is a cosmopolite mosquito, vector of arbovirosis. The worldwide studies of its insecticide resistance have demonstrated a strong loss of susceptibility to pyrethroids, the major class of insecticide used for vector control. French oversea territories such as French Guiana (South America), Martinique and Guadeloupe islands (Lesser Antilles) as well as New Caledonia (Pacific Ocean), have encountered such resistance. In 2009, we initiated a research program on the pyrethroid resistance in French Guiana, Guadeloupe and New Caledonia. *Ae. aegypti* populations were tested for their deltamethrin resistance level then screened by an improved microarray developed to specifically study metabolic resistance mechanisms. Cytochrome P450 genes were implicated in conferring resistance. CYP6BB2, CYP6M11, CYP6N12, CYP9J9, CYP9J10 and CCE3 genes were upregulated in the studied populations and were common to other populations at a regional scale. The implication of those genes in resistance phenomenon is then strongly suggested. Other detoxifying genes were also upregulated at lower scale. These first results were complemented by screening for target site mutations on the sodium channel voltage dependent gene (eg. kdr). The V1014I mutation on the DIIS6 was observed in the population from French Guiana and Guadeloupe. After establishing resistance backgrounds we will undertake further research on the relationship between the environment and the profile of resistance. Ecological and anthropogenic observations, results of field and lab experimentations, and vector control practices will be related to mosquito genomic and transcriptomic profiles that will help us to better understand the dynamics of resistance.

## 385

### THE DURABILITY OF LONG-LASTING INSECTICIDE-TREATED NETS IN ZAMBIA - BASELINE RESULTS FROM A TWO-YEAR PROSPECTIVE COHORT STUDY

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Long-lasting insecticide treated nets (LLIN) are a key malaria intervention in sub-Saharan Africa. Estimating net longevity and need for replacement is critical for long-term malaria control. Manufacturers report that LLINs last 3-5 years. However, an unpublished study in Zambia suggests that LLINs physically deteriorate before 27 months. In 2012, the President's Malaria Initiative (PMI) collaborated with the United States Peace Corps on an ongoing net durability study in Zambia. With assistance from the National Malaria Control Center, staff from the PMI and the Peace Corps Malaria Coordinator trained Peace Corps Volunteers and local Zambian counterparts in interviewing and data collection. The study examines physical integrity and insecticide persistence in LLINs over time. LLINs distributed in two different provinces, Permanets® (Luapula Province) and Olysets® (Northern Province), are followed every 6 months to count and measure holes (size approximated by thumb, fist, and head) for 2 years. Households are surveyed about net care and usage. We present information on the physical durability of 12-month-old nets. We enrolled 999 LLINs; 505 Permanets® and 494 Olysets®. Of the 999 households, 91.9% of those interviewed used the study netthe night before the interview. 71.0% of nets were previously washed, and Permanets® were slightly more likely than Olysets® [RR 1.08 (1.02-1.15)] to have been washed. LLINs were hung most commonly over reed mats (24.3%); households in Luapula Province were less likely to use reed mats [RR 0.73, CI (0.56-0.95)]. About one-third of the nets had holes of any size (n=307, 30.7%), with no difference between type of net; 34.4% of Permanets® vs 34.8% of Olysets® [RR 0.97, CI (0.89-1.05)]. The proportions of the size of holes did not differ between types of nets. Nets hung over reed mats were more likely to have holes [RR = 1.37, CI (1.06-1.78)]. In sum, nets are not as durable as expected, especially when hung over reed mats. A strong collaboration between the Peace Corps and the PMI makes this village-based study possible.

## 386

### LARVICIDAL ACTIVITY OF A PHOTO-ACTIVATED PORPHYRIN AND NEEM PORTION FORMULATIONS AGAINST *ANOPHELES GAMBIAE* S.L

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The spread of insecticide resistance among *Anopheles* mosquitoes raise the needs for new insecticidal malaria vector control strategies. Photo-activated porphyrins induce lethal tissue damage to organisms through oxidative stress mechanisms. Neem extracts are known to possess insect growth regulator properties on mosquito larvae. Thus, portions of neem (*Azadirachta indica*) plant could constitute candidate carriers for the meso-substituted cationic porphyrin (C12) against *Anopheles* mosquitoes. Based on this hypothesis, the larvicidal efficacy and the delayed effect of combinations of the two types of larvicidal tools were assessed on larvae of wild-caught *An. gambiae s.l.* from Burkina Faso. In outdoor trays' experiments, the C12 formulations were assayed against batches of 60 larvae treated in water samples from potential *Anopheles* larval breeding sites. Among the tested porphyrin neem formulations for efficacy, the porphyrin neem fruit combination (NF-C12) was able to induce about 82.7±6.3% mortality after 44h of exposure irrespective of breeding site water type of treatment. However, while favoring a rapid killing effect against mosquito larvae, the combination porphyrin neem reduced by about half the delayed effect of neem portion against *An. gambiae s.l.* larvae. In the contexts of management of integrated vector control and resistance management, the neem fruit used as C12 porphyrin carrier may constitute a promise larvicidal tool against *An. gambiae s.l.*.

## 387

### CHARACTERIZATION OF BACTERIAL DIVERSITY IN THE MIDGUTS OF WILD *ANOPHELES GAMBIAE* MOSQUITOES AND THE IMPACT OF NATURAL MIDGUT BACTERIAL COMMUNITIES ON *PLASMODIUM FALCIPARUM* SPOROGONIC DEVELOPMENT

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Malaria remains the most impactful vector-borne disease worldwide and a number of methods used to control the disease are focus on combined interventions, among them the insecticide-treated nets (ITNs) and treatment with effective antimalarial drugs. Despite these methods some resistance have been observed and due to unavailability of an effective vaccine, a recent studies now focus on tripartite interactions between vectors, parasite and the vector's intestinal microflora at the molecular level have revealed complexities that can drastically affect immune responses and *Plasmodium* densities in mosquitoes. Now the influence of environmental factors on the *Plasmodium* transmission success have been study, we present here the diversity of natural migdut bacteria at different stage of *Anopheles gambiae* population in four localities in Yaounde Cameroon. Bacterial communities of wild *An. gambiae* mosquitoes was recovered using a conventional culture technique on MacConkey medium and sequencing using the 16S rRNA gene. Interestingly, the results gut community revealed that the enterobacteriaceae family was dominant in all developmental stage and the main genera were Escherichia-shigella and Serratia with 55% and 38% respectively in adult female mosquitoes. This diversity was previously described except here where we report the presence of *Delftia* genus in *Anopheles* mosquito; the genus Enterobacter was found at larval stage and adult males and this is in contrast with others studies. We next measured the effects of these natural bacterial isolates to *Plasmodium falciparum* infection prevalence and intensity over multiple infectious feedings using a meta-analysis. Our investigations to verify the potential role of field isolated bacteria shown that the prevalence and intensity of *Plasmodium falciparum* infection was drastically reduced when mosquitoes were first challenged with *Pseudomonas stutzeri, Serratia marcescens* and *Escherichia coli* whereas *Enterobacter* sp has no detectable effect which is contrast with the recent study. The details of natural mosquito gut and their effects on natural *Plasmodium falciparum* are now study but the mechanisms used by bacteria remain poorly elucidated. Deciphering microbe-pathogen interactions remains the challenge and may offers new perspectives to control malaria transmission.

## 388

### BITING PATTERNS AND SEASONALITY OF *ANOPHELES GAMBIAE SENSU LATO* AND *ANOPHELES FUNESTUS* GROUP UNDER PROLONGED USE OF INSECTICIDE-TREATED BED NETS IN KAMULI DISTRICT, UGANDA

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We investigated the biting patterns and seasonal abundances of *Anopheles gambiae s.l.* and *An. funestus* mosquitoes under prolonged use of insecticide-treated bed nets (ITNs) in Kamuli District, Uganda. The number of mosquitoes caught biting humans, the indoor and outdoor human biting densities and rates during different hours of the night, and mosquito abundances for the twelve-month sampling period in both intervention and non-intervention zones are being reported. Hourly indoor and outdoor catches of human biting mosquitoes were sampled from 19.00 to 07.00 hours for four consecutive nights each month using bed net traps in forty-eight houses randomly selected from five intervention villages and five non-intervention villages. The indoor and outdoor human-biting fractions, time of biting of the anophelines and climatic data were recorded from January to December 2010. Approximately four times more *Anopheles* mosquitoes were caught biting humans in the non-intervention zone than in the intervention zone, with *An. gambiae s. l.* catches exceeding those of *An. funestus*. In both zones, peak night biting occurred between 23.00 and 05.00 hours, indicating that ITNs should be effective. The majority of bites occurred between 03.00 and 06.00 hours for both *An. gambiae s. l.* and *funestus* group. Outdoor biting densities of *An. gambiae s. l.* exceeded the indoor biting densities throughout night in both zones, while the indoor and outdoor human biting densities of *An. funestus* group were apparently equal. The outdoor and indoor human biting rates were similar in both zones. In the intervention zone, the abundance of *An. gambiae s.l.* was rainfall-dependent, while the *An. funestus* group could shrive with or without rain fall. In the non-intervention zone, both *An.gambiae s.l.* and *An.funestus* mosquitoes shrived all year round regardless of the amount of rainfall. Considering the effectiveness of ITNs and biting patterns and seasonal abundances exhibited by *An. gambiae s.l.* and *An. funestus* mosquitoes in Kamuli district, scaling up the use of Long Lasting ITNs (LLINs) in combination with indoor residual spraying, environmental management and improved house designs in the context of integrated vector management may be the appropriate vector control strategy.

## 389

### MODULATION OF *ANOPHELES* GENE EXPRESSION BY NITROQUINE

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Antimalarial drugs may impact mosquito's defense against *Plasmodium* parasites. Our previous study showed nitroquine significantly reduced infection of *Anopheles stephensi* by *P. yoelii*, but the underlying mechanism remains unclear. In order to understand how transmission capacity of *An. stephensi* was affected by nitroquine, we explored the transcriptome of adult females after different treatments and examined changes in gene expression profiles. As the *An. stephensi* genome has not yet been published, we adopted a new method to identify process-related genes based on their differential expression.We extended massively parallel sequencing and data analysis (including gene discovery, expression profiling, and function prediction) to *An. stephensi* before and after *Plasmodium* infection with or without nitroquine treatment. Using numbers of reads assembled into specific contigs to calculate relative abundances (RAs), we categorized the assembled contigs into four groups according to the differences in RA values: infection induced, infection suppressed, drug induced, and drug suppressed. We found both nitroquine in the blood meal and *Plasmodium* infection altered transcription of mosquito genes implicated in diverse processes, such as immunity (*e.g.*, pattern recognition receptors, extracellular signal modulators, and intracellular signal transducers), cytoskeleton, adhesion, and oxidative stress. The differential gene expression may have promoted defense responses of *An. stephensi* against the parasite and thus decrease its infectivity. Our study indicated that nitroquine may regulate several immune mechanisms in the mosquito against *Plasmodium* at the level of gene transcription. This highlights the need for better understanding of antimalarial drug's impact on parasite survival and transmission. In addition, our data largely enriched the existing sequence information of *An. stephensi*, an epidemiologically important vector species.

## 390

### HUMAN ANTIBODY RESPONSE TO *AEDES AEGYPTI* SALIVARY PEPTIDE AS A NEW INDICATOR FOR ASSESSING THE RISK OF ARBOVIRUSES TRANSMISSION AND THE EFFICACY OF VECTOR CONTROL, IN DENGUE AND CHIKUNGUNYA TRANSMISSION AREAS

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New indicators are urgently needed to assess the human exposure to *Aedes* mosquito bites and therefore, to evaluate the risk of dengue (DENV) and chikungunya virus transmission, and the efficacy of vector control strategies. Previous studies have demonstrated that human antibody (Ab) responses to *Aedes aegypti* Nterm-34kDa salivary peptide could represent promising tool for evaluating the human-*Aedes* contact. In order to validate this indicator, we investigated its usefulness for measuring both, the risk of arboviruses transmission and the efficacy of vector control. Specific IgG response to Nterm-34kDa peptide was evaluated from 208 individuals, within a cross-sectional study conducted in urban area of Vientiane city in Laos, Southeast Asia. The specific IgG responses in individuals living in low urbanized neighbourhoods were higher than those from the high urbanized ones ($P < 0.0001$). A similar pattern was observed concerning the prevalence of recent DENV infection. This suggests that the exposure of vectors bites, and therefore the risk of DENV transmission, is probably higher in low urbanized areas than in the more urbanized ones. Human IgG response to Nterm-34kDa peptide was also assessed from 102 individuals living in urban area of Saint-Denis in La Reunion Island, Indian Ocean, before and after the implementation of vector control against *Aedes* mosquito. Specific IgG response decreased just since 2 weeks ($P < 0.0001$), and until 4 weeks post-intervention ($P = 0.0002$). This decrease appeared, during the first week post-intervention, to be associated to the decline of *Aedes* mosquito density, estimated by entomological parameters. This indicates a probable earlier but not longer reduction of exposure to *Aedes* bites after vector control implementation. Altogether, these results showed that human IgG Ab response to *Aedes aegypti* Nterm-34kDa salivary peptide could be a pertinent indicator for, i) predicting areas with higher risk of arboviruses transmission and, ii) evaluating the efficacy of vector control programs.

## 391

### MOSQUITO AND FLAVIVIRUS SURVEILLANCE FROM FLOOD AFFECTED AREAS IN THAILAND

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Over 10% of arable land was destroyed and 815 deaths resulted from the disastrous flooding of 2011 in Thailand. Water levels reached waist height in some areas of Bangkok by late October and remained elevated well into January 2012. The threat of vector-borne disease, such as Japanese encephalitis or dengue, is always a concern in high-density populations and Bangkok is no exception. We surveyed mosquito populations using $CO_2$ - baited, CDC light traps in 7 flood affected areas in Bangkok and nearby Pathumthani province during and immediately after the flooding. Our goal was to identify community composition, abundance, and the presence of flavivirus in collected mosquitoes. A total of 110,510 and 124,588 mosquitoes were trapped during and after the flood, respectively. Our sample consisted of 8 genera: *Culex sp., Mansonia sp., Aedes sp., Anopheles sp., Lutzia sp., Armigeres sp., Coquillettidia sp.* and *Uranotaenia* species. A total of 18 species were captured during the flood and 22 species were collected after flood waters had begun receding. *Culex* mosquitoes, the most abundant specimens, were pooled by species and location and randomly selected for flavivirus RNA screening using SYBR Green I-based real-time RT-PCR assay. All 217 tested pools (6,905 mosquitoes) of *Cx. tritaeniorhynchus, Cx. vishnui,* and *Cx. gelidus* were negative for flavivirus. This study provides a better understanding of mosquito diversity, abundant, and mosquito-transmitted flavivirus situation from flood affected areas in Thailand.

## 392

### GENETIC BASIS OF VECTOR COMPETENCE FOR FIELD DENGUE VIRUS ISOLATES IN A NATURAL POPULATION OF *AEDES AEGYPTI*

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Successful infection and dissemination in the mosquito vector are prerequisites for dengue virus (DENV) transmission among humans. The ability of a mosquito to become infected following ingestion of infectious blood and to subsequently develop a disseminated infection varies substantially between and within populations of the primary DENV vector, *Aedes aegypti*. Knowledge of the genetic basis of *Ae. aegypti* vector competence for DENV mainly derives from laboratory-tractable systems that consist of artificially selected mosquito lines and a single reference DENV strain. DENV exists in nature as four antigenically distinct serotypes with considerable intra-serotype genetic diversity. We characterized the genetic basis of vector competence for different DENV isolates from two serotypes in a natural *Ae. aegypti* population. Mosquito isofemale families were derived from wild eggs collected in Kamphaeng Phet, Thailand. Families were experimentally exposed to low-passage DENV isolates obtained from the serum of human patients. We used a quantitative genetic approach to survey genetic factors controlling vector competence based on both inter- and intra-family variation. Analysis of inter-family variation revealed that mosquito genetic factors strongly influence natural vector competence in this *Ae. aegypti* population. Analysis of intra-family variation allowed us to locate some of these factors in the *Ae. aegypti* genome by genetic mapping. Importantly, whereas some mosquito genetic factors had a generalist effect across DENV serotypes and isolates, others acted in a virus isolate-specific manner. Thus, multiple genetic factors control vector competence for DENV in this natural *Ae. aegypti* population but the effect of these factors is modulated by the viral genome. Our results demonstrate that DENV transmission is controlled by a complex interaction between the mosquito and viral genomes. They emphasize the importance of taking into account viral genetic diversity for understanding the genetic basis of mosquito vector competence in natural populations.

## 393

### ROLE OF NUTRITIONAL RESERVES AND BODY SIZE IN *ANOPHELES GAMBIAE* MALES MATING SUCCESS

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A better knowledge of the different parameters that account for male mating success in the wild is critical to the development of genetic control strategies. In this study, we measured energy budgets (total sugar and glycogen) as the daily energetic investment in swarming males of *Anopheles gambiae s.s.* M and S molecular forms from two different field locations, VK7 and Soumousso. We also looked at the difference between energetic reserves in mated males compared to unmated ones, and assessed wing length in both molecular forms to explore whether this phenotypic trait was involved in swarming behavior or mating success. The current study showed that the energetic cost of 25 minutes of swarming was around 50% of the male's sugar (M form: 48.54%, S form: 56.27%) and glycogen (M form: 53.13%, S form: 59.04%) reserves. However, no difference in carbohydrate content was observed between mated and unmated males. Mated males were found to be bigger than unmated ones, while intermediate size of males is advantageous in mating system, both in M and S molecular forms and when collected in two different locations. Regardless of the collection location, no difference in wing size was observed in swarming males collected early or late during a particular swarm. The results are discussed in the context of ecological and sexual selection.

## 394

### ASSESSING TRANSMISSION OF LYMPHATIC FILARIASIS IN ENDEMIC COMMUNITIES WITH AT LEAST FIVE ROUNDS OF MASS DRUG ADMINISTRATION IN GHANA

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There has been a critical question of when to stop Mass Drug Administration (MDA) in lymphatic filariasis (LF) endemic communities. After at least five (5) rounds of MDA with ivermectin and albendazole, it is expected that microfilaraemia should reach a minimum threshold in the human population such that the vectors (predominantly *Anopheles gambiae*) may not be able to pick up microfilaria during a blood meal. In Ghana, studies have indicated that some vectors particularly An. melas and Mansonia spp could transmit at low levels of microfilaraemia thus keeping a residual transmission of the disease even after more than seven rounds of LF MDA. Using Pyrethrum Spray Catches (PSC), mosquitoes were sampled from Ayensuako, Gyaahadze, and Mankrong communities where more than 5 rounds of MDA have been done. All mosquitoes caught were morphologically identified and dissected for infection with W. *bancrofti* and the cibarial armature examined for the various species. A total of 550 mosquitoes were collected. Distribution of mosquitoes in these endemic communities was predominantly *An. gambiae s.l.* - 462/550 (84.0%). The proportion of other mosquitoes species were An. *funestus* 9/550 (1.6%), An. pharoensis 1/550 (0.2%), *Culex* sp 57/550 (10.4%). and Mansonia *sp.*, 21/550 (3.8%). For all samples, microscopy was negative for LF parasites. In general, the cibarial teeth of the *Anopheles* species were significantly more than those observed for *Mansonia sp.* and for *Culex sp.* Although

the numbers of mosquitoes collected were low due to the period of collection, the results compared to similar studies in the same region of Ghana indicate that MDA in this area has possibly led to elimination of transmission. The impact of this observation and the analysis of the cibarial armature for the different species are discussed.

## 395

### VECTOR COMPETENCE OF *AEDES ALBOPICTUS* AND *AE. AEGYPTI* TO TWO DIFFERENT CHIKUNGUNYA STRAINS IN THAILAND

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September 2008 saw the beginning of a massive outbreak of chikungunya fever along the Thai-Malaysia border. Over 49,000 cases were reported before this outbreak was finished in late 2009. The recognized vector for chikungunya virus (CHIKV) is the mosquito, *Aedes aegypti*, however *Ae. albopictus* has been recently implicated as the principle vector in several countries. Our goal was to determine the vector competence of both *Aedes* species (AFRIMS lab strain) to two different CHIKV strains; CHIKV historical strain isolated in 1979 and the epidemic strain isolated in 2009. We assessed infection and dissemination rates from orally infected, female mosquitoes at 7 and 14 days post-infection using the SYBR Green I-based real-time RT-PCR. Of the 2 species, CHIKV was only detected in *Ae. albopictus*. In this species, we found over 6 time higher infection and dissemination rates with the 2009 epidemic strain of CHIKV compared to the 1979 historical strain. Our results support previous studies suggesting that *Ae. albopictus* is a capable vector of CHIKV and could easily been responsible for the recent outbreak of chikungunya fever in southern Thailand.

## 396

### TEMPORAL VARIATION IN EXPOSURE TO *ANOPHELES GAMBIAE* AND RISK OF MALARIA TRANSMISSION

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Human biting rates and mosquito infection rates vary across space and time. Mosquito populations also vary temporally, forced by environmental variables such as rainfall, temperature, and humidity. These sources of heterogeneity in the distribution of mosquito populations generate variability in the risk of human infection. Assessment of exposure to malaria vectors is important to our understanding of disease transmission risk, and facilitates planning of control efforts. *Anopheles gambiae* salivary peptide (gSG6-P1) has been designed to enhance its specificity and immunogenicity to detect human exposure to malaria vectors. We evaluated total IgG responses to gSG6-P1 and two malaria antigens (CSP, MSP-119) in an age stratified cohort (< 5, 5-9, >9) from Asutuare, South-western Ghana, an area of relatively low but perennial transmission. 200 randomly selected sera were analyzed from archived samples belonging to a cohort that were followed at 3 contact times (n = 600) as follows; February, toward the end of the dry season, May at the peak of the major rainy season and August a dry period before the minor rainy season representing snap shots of the perennial transmission in the year. Seropositivity above threshold of negative group to the 3 antigens was detected in the cohort at all contact points across age groups. Although, seroprevalence showed temporal trends similar to rainfall and mosquito exposure patterns, specific responses to MSP 1 and CSP, Chi square analysis did not yield significant differences among the different time points. MSP 1 19; 53.3%, 60.6%, 56.7% (chi2= 1.91, p = 0.38), CSP; 21.7%, 19.4% and 23.3% (Chi2 1.53, p =0.46) in Feb, May and August in respective order. In contrast to the above, analysis of seroprevalence and median antibody levels to gSG6-P1 showed significant differences, detecting temporal variations in vector exposure among the cohorts at different time points. Where gSG6 - P1, from Feb, May and August respectively were; 46.2%, 49.7% and 35.7%, (chi2= 7.41, p= 0.02). Repeated measures ANOVA as well as post hoc Tukey multiple comparison test showed significant difference in antibody levels in mosquito exposure between the peak rainfall and dry period preceding minor rainy season. It is concluded that gSG6-P1 is robust and sensitive to detect temporal changes in human exposure.

## 397

### THE RELATIONSHIP OF LAND COVERS WITH ANTHROPOPHILIC *ANOPHELES* SPECIES DIVERSITY AND COMPOSITION IN TWO MALARIA ENDEMIC REGIONS OF COLOMBIA

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The influence of land cover on the abundance, composition and diversity of anthropophilic anophelines was evaluated in six localities of two Colombian malaria endemic regions, the Urabá-Bajo Cauca-Alto Sinu (UCS) and Pacific (PAC) regions, from November 2008 to June 2010. Also, for each site, supervised classification of the types of land cover was performed using the land cover diversity index (SHDI), satellite imagery Landsat7-TM and ground-truthed data. A total of 9,839 specimens were collected that corresponded to 10 species. *Anopheles darlingi* and *Anopheles* nuneztovari were the most abundant species (47.21% and 40.47%, respectively). There was a significant negative relationship between anopheline species diversity and land cover types. The analyses showed localities with high anopheline diversity and low SHDI and others with reduced anopheline diversity and high SHDI. In particular, land cover diversity, type of coverage and pluviosity strongly influenced the distribution of anopheline communities. The presence of An. nuneztovari was correlated with grass cover and bare soils, while An. *darlingi* was correlated with forested cover. These results indicated that the diversity in land cover and some climatic variables contributed to the observed variation in anopheline community structure. This information can be used for the design of more specific vector control strategies in malaria endemic regions of the country.

## 398

### MALARIA VECTOR BIOMICS IN MUTASA DISTRICT, ZIMBABWE

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In December 2012, an International Centers of Excellence in Malaria Research (ICEMR) project began in Mutasa District, Zimbabwe. Recently, Mutasa has experienced a surge in malaria cases despite the use of insecticide treated bed nets (ITNs) and other control efforts. Preliminary research using morphological identification of mosquitoes obtained through human landing catches and CDC light traps suggests that the primary malaria vector in the region is *Anopheles funestus* senus lato. Our aims are to elucidate the true nature of the vector biomics in regard to malaria transmission in Mutasa District, Zimbabwe. Molecular diagnostics to accurately identify vector species, blood meal analysis to determine feeding behavior, and insecticide resistance assays will be utilized to

examine the role of these mosquitoes in malaria transmission. The role of mosquitoes in the transmission of malaria is an important component in eventual control and elimination of the disease.

## 399

### LONG TERM IMPACTS OF COMBINED SEWER OVERFLOW REMEDIATION ON WATER QUALITY, MOSQUITO POPULATION DYNAMICS AND WEST NILE VIRUS AMPLIFICATION

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Combined sewer systems are a significant source of urban water pollution due to the overflow of minimally treated sewage into natural streams (aka a combined sewer overflow, CSO). CSOs contribute to the impairment of natural waterways and are also associated with increased mosquito productivity and elevated risk of West Nile virus (WNV) transmission. We longitudinally investigated the impact of CSOs on water quality, immature mosquito productivity and WNV infection in the city of Atlanta, Georgia, one year before and four years after CSO-facility remediation. Water quality (ammonia, phosphate, nitrate and dissolved oxygen concentrations), immature and adult mosquitoes, WNV infection in mosquitoes, water temperature and rainfall were quantified biweekly between June-October at two urban creeks during 2008-2012. Generalized estimating equations quantified the factors explaining the elevated mosquito productivity at CSO streams and the long term impacts of CSO facility remediation on mosquito productivity and WNV amplification. Nutrient concentrations and late immature (IV-instar and pupae) mosquito populations were significantly higher in CSO than in non-CSO creeks. CSO facility remediation significantly improved all water quality estimates and reduced the number of overflows, mosquito productivity and the overall contribution of CSO-affected streams as sources of WNV infected mosquitoes. The quality of water in CSOs provided a suitable habitat for immature mosquitoes. Remediation of the CSO facility through the construction of a deep storage tunnel improved water quality indices and reduced the productivity of mosquito species that can serve as vectors of WNV.

## 400

### COMPARISON OF THE IFAKARA TENT TRAP AND THE HUMAN LANDING CATCH FOR MOSQUITO COLLECTION IN A SUDAN SAVANNAH AREA OF MALI

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Assessment of *Wuchereria bancrofti* transmission rates by vector sampling is a crucial component of mass drug administration programs to eliminate lymphatic filariasis in Africa. The most common mosquito collection method in current use, Human Landing Catch (HLC), is associated with ethical issues. We have previously reported on the suitability of the ITTC (Ifakara Tent Trap type C) as an alternative method for sampling filariasis vectors in Mali, particularly in settings where *Anopheles* vector density is high.To further assess the utility of ITTC, additional testing was performed in areas of high and low vector density in 2 villages of the Sikasso District in Mali. Mosquitoes were collected every month at three different sites per village from July to December, 2012. The sites in each village were ≥100 meters apart, and the three methods were implemented concomitantly at each site with one ITTC trap and one HLC unit that consisted of one room with two collectors- one inside and the other outside the room. The total number of *Anopheles* collected by HLC was 4,149 in 2012; this was similar to the number collected using the ITTC (3,580 *Anopheles* collected; p=0.094, Mann-Whitney). The numbers of mosquitoes captured using each of the two collection methods in 2012 were highly correlated in Bougoula (r=0.83; p=0.042; Spearman rank), Boundioba (r=0.94; p=0.0048) and when the villages were considered together (r =0.83, p=0.042). In contrast, in 2011 in Boundioba, at a time when the vector density was low (only 251 anopheles were collected by HLC as compared to 682 in 2012), the correlation between the HLC and the ITTC was not significant (r=0.61; p=0.19). From a simple linear regression using the monthly collection data from both years, the HLC yield for *Anopheles* was estimated at [11.04±37.28] + [(1.15± 0.11) x ITTC yield]. This model had a slope that significantly deviated from zero ($p<10^{-3}$) and $r^2=0.84$. In conclusion, when vector density is high, yields from ITTC collection can be used to estimate yields from HLC, allowing comparison of new datacollected without risk to collectors, to historical data.

## 401

### SOMATIC *WOLBACHIA* DENSITIES ARE LOW AND VARIABLE AND UNLIKELY TO INCREASE HOST RESISTANCE TO WEST NILE VIRUS INFECTION IN FIELD POPULATIONS OF *CULEX QUINQUEFASCIATUS* AND *CX. PIPIENS*

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The endosymbiotic bacteria *Wolbachia pipientis* infects a wide variety of insect species and can cause both reproductive phenotypes and increased viral resistance in its host. *Wolbachia* naturally infects *Culex quinquefasciatus* and *Cx. pipiens*, mosquitoes that are important vectors of West Nile virus (WNV). The native *Wolbachia* infections in these mosquitoes could potentially increase the mosquito's resistance to viral infection and thereby reduce vector competence. We recently demonstrated that native *Wolbachia* infections increase host resistance to WNV infection in laboratory colonies of the fruit fly *Drosophila melanogaster* and in *Cx. quinquefasciatus*, reducing the ability of *Cx. quinquefasciatus* to transmit WNV. Using quantitative PCR, *Wolbachia* densities were measured in the ovaries and somatic tissues of individual flies and mosquitoes from laboratory colonies and from field populations of *Cx. quinquefasciatus* and *Cx. pipiens*. *Wolbachia* densities were significantly higher in *D. melanogaster* than in *Cx. quinquefasciatus*, consistent with *Wolbachia* density determining the relative strengths of WNV resistance in these two species. *Wolbachia* densities in somatic tissue in field populations of *Cx. quinquefasciatus* and *Cx. pipiens* were significantly lower than the densities in the laboratory colony of *Cx. quinquefasciatus* originally used to demonstrate *Wolbachia*-induced resistance to WNV, and somatic *Wolbachia* densities equivalent to those measured in field populations did not inhibit infection by WNV. These results suggest that native *Wolbachia* infections in field populations of *Cx. quinquefasciatus* and *Cx. pipiens* are too low to increase host resistance to viral infection and are unlikely to reduce the competence of these mosquitoes to transmit WNV.

## 402

### GENETIC CONTROL OF *AEDES AEGYPTI*

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Mosquito-borne diseases, such as dengue fever, chikungunya and malaria, are major and increasing international public health concerns. The two main vectors of dengue are *Aedes aegypti* and *Ae. albopictus*, both notoriously difficult to control with current methods. We are developing genetic control tools to augment current methods, especially RIDL, an advanced derivative of the classical Sterile Insect Technique (SIT). In a RIDL

control programme 'sterile' male mosquitoes (male mosquitoes do not bite or transmit disease) are released continually over a wide area to mate with the target pest population; death of progeny due to inheritance of the RIDL transgene leads to decline of the target population. A key advantage is that the method is 'self-limiting'; making it controllable, reliable and reversible, in contrast to methods where the genetic change needs to persist in the wild population. Trials in the Cayman Islands in 2009 and 2010 proved the technology could reduce an *Ae. aegypti* population (80% reduction in ovitrap index despite ongoing immigration from adjacent areas). Further trials in 2011 and 2012 in Brazil provided equally positive results in very different ecological and social settings - 80% reduction in ovitrap index and even higher reduction in adult presence in a non-isolated high-density urban area and effective elimination in a more isolated village. Simulation modelling, as reported previously, indicates that even 80% suppression would be sufficient to prevent epidemic dengue in many transmission settings. This Brazilian programme is now expanding. With the smaller scale experiment conducted in Malaysia in 2010, releases in these three countries have confirmed the suitability of the modified males in terms of survival, dispersal, mating competitiveness against their wild counterparts and overall improved fitness compared to irradiated mosquitoes. Knowledge gaps include how best to integrate this genetic method with current approaches in an optimized integrated vector management system.

## 403

### OVERT DIABETES MELLITUS AMONG NEWLY DIAGNOSED UGANDAN TUBERCULOSIS PATIENTS: A CROSS SECTIONAL STUDY

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There is a documented increase of diabetes mellitus in Sub Saharan Africa, a region where tuberculosis is highly endemic. Currently, diabetes mellitus is one of the recognised risk factors of tuberculosis. No study has reported the magnitude of diabetes mellitus among tuberculosis patients in Uganda, one of the countries with a high burden of tuberculosis. This was a cross-sectional study conducted among 260 consenting adult patients with a confirmed diagnosis of tuberculosis admitted on the pulmonology wards of Mulago national referral and teaching hospital in Kampala, Uganda to determine the prevalence of diabetes mellitus and associated clinical factors. Laboratory findings as well as the socio-demographic and clinical data collected using a validated questionnaire was obtained. Point of care random blood sugar (RBS) testing was performed on all the patients prior to initiation of anti tuberculosis treatment. Diabetes mellitus was diagnosed if the RBS level was ≥ 200mg/dl in the presence of the classical symptoms of diabetes mellitus. The prevalence of diabetes mellitus among the admitted patients with tuberculosis was 8.5%. Only 5 (1.9%) patients with TB had a known diagnosis of diabetes mellitus at enrollment. Majority of the study participants with TB-DM co-infection had type 2 diabetes mellitus (n=20, 90.9%). At bivariate analysis, raised mean ALT concentrations of ≥80 U/L were associated with DM (OR-6.1, 95% CI 1.4-26.36, p=0.032) and paradoxically, HIV co-infection was protective of DM (OR-0.32, 95% CI 0.13-0.79, P=0.016). The relationship between DM and HIV as well as that with ALT remained statistically significant at multivariate analysis (HIV: OR- 0.17 95%CI 0.06-0.51, p=0.002 and ALT: OR-11.42 95%CI 2.15-60.59, p=0.004). This study demonstrates that diabetes mellitus is common among hospitalized tuberculosis patients in Uganda. The significant clinical predictors associated with diabetes mellitus among tuberculosis patients were HIV co-infection and raised mean serum alanine transaminase concentrations.

## 404

### PREVALENCE OF *LEGIONELLA PNEUMOPHILA* ANTIBODIES IN DENTAL PRACTITIONERS, 2002-2009

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This is an analysis of the association between the presence of *Legionella pneumophila* antibodies and characteristics of dental hygienists, assistants, and dentists. *L. pneumophila* antibodies were determined via serum assay. All other information was collected via self-administered paper survey. Bivariate analysis was performed to investigate the association between *L. pneumophila* antibodies and characteristics. Multivariate logistic analysis was used to control for possible confounders and effect modifiers. Geographical location, time spent practicing dentistry per year, age, and race were statistically significant predictors of variation of *Legionella* antibody seroprevalence. Antimicrobial methods utilized on dental unit water did not predict *Legionella* antibody prevalence, nor did the frequency with which dental unit water quality was monitored. This suggests that the water quality monitoring and infection control procedures currently in use by dental practitioners may not be sufficient to prevent *L. pneumophila* growth in dental unit water lines.

## 405

### TUBERCULOSIS DRUG RESISTANCE AND THE UTILITY OF GENEXPERT SYSTEM IN AN URBAN SETTING IN INDIA

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India has the highest burden of Tuberculosis cases globally and a third of the population has latent TB infection. Diagnosis of tuberculosis using conventional methods such as sputum microscopy has always been a challenge, and low rates of detection contribute to the emergence and increase in drug resistance. Recent reports have suggested that the resistance to rifampicin, which is highly correlated with multi-drug resistance, ranges from 20-74% in urban centers such as Delhi and Mumbai in India. The availability of the GeneXpert system has made it easier to diagnose TB and detect drug resistance to rifampicin at the same time. In this study, we compared the results of the GeneXpert TB system with sputum microscopy and assessed the prevalence of resistance to Rifampicin among 170 consecutive patients presenting with symptoms suggestive of TB in Bangalore, India. The mean age of the patients was 39 (±11) years and 77% were male. 16% of the patients reported having received treatment for TB previously, 5% were HIV-infected, and 8% also had diabetes. Compared with the GeneXpert system (n=112), sputum microscopy detected 20% fewer patients (95% CI: 13%, 28%) with TB. We also found that only 5% of the patients had a resistance to rifampicin using the GeneXpert system. Our results suggest that the GeneXpert system outperforms sputum microscopy even in a large urban center in India. The results also indicate that the epidemic of tuberculosis and drug resistance varies in different parts of the country, highlighting the need for active epidemiological surveillance and detection of hot spots using geographic information systems to direct and maximize utilization of limited resources.

## 406

### CHARACTERIZING CROSS-RESISTANCE TO FLUOROQUINOLONES IN *MYCOBACTERIUM TUBERCULOSIS*

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The global control of tuberculosis (TB) has been complicated by the global HIV epidemic, the increasing prevalence of multidrug-resistant (MDR) TB and the emergence of extensively drug resistant (XDR) TB. The fluoroquinolones (FQ), levofloxacin (L), gatifloxacin (G) and moxifloxacin (M), are the most effective drugs for the treatment of MDR TB. However, cross resistance between ofloxacin (O) and the more potent, later generation FQs, L, G and M, has not been defined. Definition of cross-resistance patterns will play a crucial role in maximizing the potential of FQs in the treatment of MDR and XDR TB patients, monitoring resistance trends, preventing the emergence of resistance, contributing to the discovery of new anti-tuberculous agents and improving the structure of novel FQs. This study aims to determine the relationship between cross-resistance among FQs and specific mutations in *gyrA/B*. The Quinolone resistance determining regions of *gyrA* and *gyrB* of 109 consecutive FQ resistant clinical *Mycobacterium tuberculosis* isolates collected from Pham Ngoc Thach Hospital TB reference laboratory for Vietnam have been sequenced. Mutations in these regions account for over 80% of resistant clinical isolates from Vietnam. 49 representative isolates of this collection including nine novel gyrB mutants were characterised for their minimum inhibitory concentration (MIC) for O, L, G and M on automated liquid MGIT 960™ system. Different mutations showed variation in the MIC to the 4 tested FQs. Non-*gyrA/B* mutants showed borderline MICs to all 4 drugs. Single A90V-*gyrA* mutants show intermediate MICs to O, L but very low MICs to G, M whereas combinations with other mutations in *gyrA/B* confer high MICs to all FQs. Mutations at codon 94 of *gyrA* confer high MICs while variation in MIC level was observed dependent on the amino acid change at this site. Single novel mutations in *gyrB* show variations in their MIC level. Resistance level for FQs varies by mutation in *gyrA/B*. This project received support from the Wellcome Trust and the International Society for Infectious Diseases.

## 407

### EVALUATION OF A MOLECULAR DIAGNOSTIC PLATFORM FOR SIMULTANEOUS DETECTION OF MULTIPLE RESPIRATORY PATHOGENS IN THAILAND

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Acute lower respiratory tract infections (ALRI) are a leading cause of morbidity and mortality globally, resulting in 2 million deaths in children aged <5 years annually. Testing for multiple viruses and bacteria has historically required multiple individual assays, which can be resource intensive and delay reporting of test results. The TaqMan® Array Card (TAC) is a molecular diagnostic platform that allows simultaneous detection of 30 viral and bacterial respiratory pathogens. We tested 90 stored nasopharyngeal swab specimens from patients hospitalized with ALRI in rural Thailand in 2011. Specimens were originally tested at the Thailand National Institute of Health by real-time RT-PCR (rRT-PCR) single-plex CDC assays for influenza A and B, respiratory syncytial virus (RSV), adenovirus, and human metapneumovirus (HMPV). Specimens for TAC testing were selected from patients who originally tested positive for at least 1 of these 5 viruses (n=85) plus 5 patients that had tested negative; 34 (38%) had been positive for >1 virus. After total nucleic acid extraction, specimens were tested by TAC in parallel with rRT- PCR for these 5 viruses. Using real-time PCR as the gold standard, the sensitivities of TAC were 100% (27/27) for influenza A, 100% (18/18) for influenza B, 100% (15/15) for adenovirus, 90% (9/10) for RSV, and 87% (20/23) for HMPV. The specificity of TAC was 100%. In addition to these 5 viruses, TAC testing detected other viruses or bacteria in 54 (60%) of 90 specimens. Overall, 68 of 90 were positive for >1 pathogen, including 49 with both bacteria and viruses detected. Our findings demonstrate the high sensitivity and specificity of TAC in a field setting for 5 common respiratory viruses compared with individual rRT-PCR assays. TAC could be a useful platform for rapid and simultaneous detection of multiple pathogens in certain settings such as outbreak investigations.

## 408

### ADVERSE REACTIONS OF ANTI-TUBERCULOSIS DRUGS: A RETROSPECTIVE STUDY IN PHAM NGOC THACH HOSPITAL

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Adverse drug reactions (ADRs) due to anti-tuberculosis therapy can affect on tuberculosis (TB) treatment adherence and outcome. In Vietnam, data of anti-TB-induced ADRs are limited. This study was undertaken to describe ADRs to antituberculous agents among in-patient admissions at a large TB reference hospital. A retrospective study reviewed 315 records of all TB patients with ADRs admitted to Pham Ngoc Thach Hospital, Ho Chi Minh City, Vietnam in 2010. Of the 315 patients admitted with suspected antituberculous therapy-associated ADRs, there were 203 (64.4%) males and 112 (35.6%) females; mean age was 46 years old; 80 (25.4%) patients had a previous history of TB treatment; 129 patients (41%) had co-morbidities including HIV-infection (46 cases, 35.7%), diabetes (20 cases,15.5%). Median time interval from initiation of TB treatment to experiencing ADRs was 27 days. The majority of detected ADRs were attributed by physicians to rifampicin (88/315, 27.9%), pyrazinamide (81/315, 25.7%), streptomycin (78/315, 24.8%). Isoniazid and ethambutol were suspected less frequently (43/315, 13,7% and 33/315, 10,5%, respectively). 37 patients (11.8%) had ADRs associated with the second line TB drugs, 90 patients (28.6%) the drugs causing ADR were not established. There were 131 patients (41.6%) had ADRs with a single TB drug, 29.8% patients had ADRs with between 2 to 6 TB drugs. The highest percentage of ADRs (149/315, 47.3%) causing hospital admission was skin reactions; following hepatitis ADRs (29.3%); then nausea, vomiting, abdominal pain (51/315, 16.2%). Anti-tuberculosis drugs were rechallenged in 177/315 (56.2%) patients with different doses, of whom 128/177 (72.3%) developed re-introduction reactions, 26/177 (14.7%) had no recurrence of ADR symptoms and the remaining (13%) had unclear reactions. 34%patients were discharged without prescription for TB therapy.In conclusion, these findings describe anti-TB-induced ADRs in a TB hospital. It is necessary to develop a prospective evidence base and a clinical aligorithm to guide clinicians in the introduction of anti-TB drugs after the development of ADR in patients with TB. This study demonstrates that current approches are highly variable and not based on good evidence.

## 409

### INCIDENCE OF RESPIRATORY TRACT INFECTIONS IS INCREASING EVEN IN AREA LOCATED 2200 METERS ABOVE SEA LEVEL IN KENYA

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Respiratory tract infections (RTIs) are the most common cause of morbidity in African countries and may be accompanied with severe complications. About 2 million of children die every year in the world and more than 40% of these deaths are attributable to Africa. In this study, we have screened clinical records of all patients treated at Ladislaus Batthanyi Strattmann Clinic and diagnosed with RTI. Diagnosis of RTI was based on clinical examination. We have noticed, that RTI were the most prevalent infection among all patients treated in abovementioned clinic, counting for 40,48% in average. However, interesting increase of prevalence of RTI was observed, with 21,41% proportion in 2009 and 30,22% in 2010, then arising above 50% in 2011 and 2012 (52,11% and 58,17% respectively). RTI are still dominating cause of morbidity among patients treated in Eldoret in Kenyan highlands, and interestingly they are becoming even more prevalent than in past. This may be assumed to better diagnostic tools, what allow physicians to differentiate RTI that would otherwise be misdiagnosed as malaria.

## 410

### RESPIRATORY VIRUSES IN CHILDREN WITH AND WITHOUT RESPIRATORY SYMPTOMS IN PERU

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Viral respiratory infections are a primary cause of illness in developing countries, disproportionately affecting young children. Some of the most widely described viral respiratory pathogens in children are respiratory syncytial virus, influenza virus, adenovirus, metapneumovirus, rhinovirus and coronavirus. Despite vast information about these respiratory viruses' characteristics in symptomatic disease, sparse data exists about their presence in those without symptoms. The aim of this study was to use real time PCR to indentify respiratory viruses in symptomatic and asymptomatic children in three large referral hospitals in Lima, Peru. We also investigated the relationship between the presence of disease and a specific virus strain. Cases were defined as individuals five years old or younger with five days or less of influenza-like illness symptoms, defined as fever (≥38°C) and one or more of the following: sore throat, cough, or runny nose. Controls were age and gender matched individuals attending the same health facility without respiratory complaints in the previous two weeks. Nasopharyngeal samples were analyzed using real time PCR and low-density array cards. Positive samples underwent sequencing for strain identification and phylogenetic analysis. Our preliminary results show that some respiratory viruses, such as respiratory syncytial virus, have a high frecuencie of detection in asymptomatic subjects, suggesting that identification of a virus does not always indicate causation of disease.

## 411

### TB PERICARDITIS: IS THERE A ROLE FOR INTERFERON-Γ RELEASE ASSAYS IN IMPROVING DIAGNOSTICS? A CASE REPORT AND REVIEW OF THE LITERATURE

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A 14 year old male Vietnamese immigrant presented with 2 weeks of fever, shortness of breath, cough, chest pain, and weight loss; having last visited South East Asia 2 years ago. He had tachycardia, jugular venous distention, pericardial friction rub, and hepatosplenomegaly. Chest x-ray showed an enlarged heart; echocardiogram revealed pericardial effusion with cardiac tamponade; and he underwent pericardiocentesis. Gram stain, aerobic/anaerobic culture, fungal stain/culture, and acid fast bacilli (AFB) stains were negative from pericardial fluid. HIV antibody screen was negative. He was discharged with a diagnosis of viral pericarditis, but was readmitted 3 weeks later due to persistence of symptoms. Tuberculosis (TB) skin test (TST) was negative, but an interferon-γ release assay (IGRA) (QuantiFERON-TB Gold) was positive. He improved following empiric TB therapy and adjunctive steroids. The diagnosis of TB pericarditis was subsequently confirmed with positive pericardial fluid culture for *Mycobacterium tuberculosis* complex, pan-susceptible. TB pericarditis is a rare manifestation of TB, uncommonly seen in the U.S. The diagnosis can be challenging, as TB may not be initially considered, and available TB diagnostics have modest sensitivity. Early diagnosis requires a high index of suspicion; especially for those with an increased background risk for TB infection. In our case, while the TST was negative, the simultaneously drawn IGRA was positive. The role of IGRAs in the diagnosis of TB pericarditis is speculative, as there is a lack of data on the sensitivity and specificity in this particular setting. It should be emphasized that TSTs and IGRAs do not lead to the actual diagnosis of TB disease, but rather heighten our index of suspicion for TB infection. The discordance between TST and IGRA in establishing latent TB infection has been increasingly recognized, and the TST-negative IGRA-positive result may simply be indicative of higher sensitivity of the IGRA. The exact immunological mechanism, however, has not been established.

## 412

### DISTRIBUTION OF HEALTHCARE RESOURCES FOR INFLUENZA PANDEMIC RESPONSE IN CAMBODIA

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Human influenza infection poses a serious public health threat in Cambodia, a country at high risk for the emergence of future pandemic influenzas. Prior pandemics, including H1N1, demonstrated the considerable adverse impact of influenza on poor communities in developing countries as well as the limitations of health system capacity for pandemic response. Investigation of healthcare resource distribution and inequalities can inform decisions regarding health resource mobilization and investment for pandemic influenza mitigation. A health facility survey was performed across Cambodia to obtain data on availability of five key resources (inpatient beds, doctors, nurses, oseltamivir, and ventilators) important for pandemic influenza response. Distributions were analyzed at the Operational District (OD) and Province levels, expanding on prior research that focused solely at the latter. A summary index of resource distribution inequality was calculated (Gini coefficient) and a potential link between socioeconomic status and resource distribution, not addressed in earlier studies, was explored by mapping resource densities against poverty rates. Gini coefficient calculation revealed variable equality in distribution of the five key resources at the Province and OD levels. A

greater percentage of the population resides in areas of relative under-supply (28.5%) than relative over-supply (21.3%). Hospital-based inpatient beds, doctors, and nurses are most heavily concentrated in wealthier areas; however, concentrations of non hospital-based inpatient beds and nurses increase alongside poverty level. The considerable heterogeneity in healthcare resource distribution across Cambodia suggests that mobilization of selected resources or patients across ODs could be beneficial in the event of a pandemic influenza. More broadly, these findings may inform future health resource investment in Cambodia, both for pandemic preparedness and general health system strengthening.

## 413

### EPIDEMIOLOGY OF CHILDHOOD PNEUMONIA AND NASOPHARYNGEAL VIRAL CARRIAGE IN RURAL NORTH PAKISTAN, APRIL 2012 - MARCH 2013

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Previous work demonstrated that pneumonia was the main cause of childhood mortality in Oshikhandass village, Gilgit, from 1989-1996. A follow-up study is on-going to determine changes in pneumonia epidemiology, response to antibiotics and carriage of viral pathogens. Female health workers trained in pneumonia management using WHO IMCI guidelines conducted weekly surveillance of children <5 years. Each episode was classified by severity and treatment or referral given. From December 2012-March 2013, nasopharyngeal (NP) swab samples obtained from children with pneumonia were tested for the presence of 20 respiratory viruses by PCR-based analysis. An average of 804 children were followed from April 2012-March 2013 with 213 episodes detected (incidence 27 /100 child years, 95% CI, 26.6-27.4, average age 22 months). Of these, 182 episodes occurred from December 2012-March 2013, (winter incidence 69/100 child years, 95% CI, 68.6-69.4); 62% of all episodes occurred in January-February. Most cases (96%) were categorized as non-severe; most children (69%) were treated with amoxicillin. Treatment failure after 3 days occurred in 10% of cases, with 1% experiencing a worsening of symptoms and 9% reporting no change; 6 needed hospitalization and none died. PCR results were available for 130 pneumonia episodes (71%). The frequency of viruses found was: enterovirus/rhinovirus (45%), respiratory syncytial virus (18%), coronavirus (229E, OC43, C44) (11%), metapneumovirus (11%), adenovirus (5%), influenza A (3%) parainfluenza 1 and 4 (3%) and bocavirus (1%). At least one viral pathogen was present in 73% of samples and mixed infections were found in 22%. We conclude that most pneumonia occurred during 2 winter months and that NP carriage of respiratory viruses is high. Public health measures to prevent spread of respiratory viruses need to be intensified just before winter season. Viral pathogens may be substantial contributors to community acquired WHO-defined pneumonia and further studies on appropriateness of antimicrobial treatment are warranted.

## 414

### *FASCIOLA HEPATICA* INFECTION IN AN INDIGENOUS COMMUNITY OF THE PERUVIAN JUNGLE

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In South America, fascioliasis is considered an illness mostly restricted to the highlands. Human fascioliasis has not been reported in the Peruvian jungle. We report 5 probable cases of autochthonous *Fasciola hepatica* infection in an indigenous population from a remote area of Manu National Park. A community intervention for soil transmitted helminths was carried out in Yomibato. Stool samples from 215 subjects were obtained and preserved in 10% formalin and 96% alcohol. These were transported to the laboratory at the Universidad Peruana Cayetano Heredia - University of Texas Medical Branch Collaborative Research Center in Cusco for parasite testing. Direct, rapid sedimentation, and Kato Katz tests were performed in all samples. Five subjects (2.3%) were identified as having eggs with the morphological appearance of *Fasciola hepatica* in the rapid sedimentation and Kato Katz tests. The mean number of eggs/gram of stools was 40 (±28.3). The mean age of the subjects was 15.5 (±6.3) years, 4 were female, 1 had significant anemia, and 2 had stunting. Four subject were co-infected with other helminths or protozoa: 2 had *Trichiuris*, 1 hookworm, 1 *Giardia*, and 2 *Blastocystis hominis*. None had a history of living in or consuming vegetables coming from the highlands. All subjects had received 4 doses of albendazole in the previous 3 months. RT- PCR with primers targeting the trematode *18s rRNA* gene and the *Fasciola hepatica ITS-1* region was performed on the stool samples. DNA from adult *Fasciola* parasites and stools from a *Fasciola* infected patient in Cusco (altitude 3,400 m) were the positive controls. In 3 subjects, the trematode *18s rRNA* PCR showed amplicons with melting curves similar to those from the positive controls. Two of these subjects also had a positive RT-PCR test for the *Fasciola hepatica ITS-1* region. This is the first report of *Fasciola hepatica* infection in 2 indigenous subjects from a remote area of the Peruvian jungle. Further epidemiological studies may be required to confirm the presence of *Fasciola* and its secondary hosts in the region.

## 415

### FASCIOLIASIS IN PAUCARTAMBO-CUSCO: SIGNIFICANT PREVALENCE VARIATION AMONG COMMUNITIES WITHIN A SMALL GEOGRAPHIC AREA

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Fascioliasis is an important problem in the highlands of Peru. Mass triclabendazole treatment is a proposed control strategy for hyperendemic areas which makes imperative knowing the prevalence of *Fasciola* at the community level. We studied the epidemiology of *Fasciola* in 6 communities in a small geographic area of Huancarani district in Paucartambo, Peru. The prevalence of *Fasciola* and intestinal parasites, anemia, and malnutrition was studied in children 3 to 12 years old receiving mass albendazole treatment. Direct, rapid sedimentation, and Kato Katz tests were performed on at least one stool sample in 290 children (Huacaycancha 23.3%, Huayllapata 19.7%, Piscohuata 16.6%, Ohuay 16.2%, Chinchayhuasi 14.8% and Queunacancha 9%). Mean age was 10 (±2.6) years, 52% were male, mean size of the household was 6.2 (±17) people, and mean school years of the mother was 3.8 (±3). For most (93%) the drinking water source is the community's reservoir, 72% own livestock, and 66% participate in livestock tending activities. Ten percent of children were underweight, 33% were stunted, 2.8% had wasting, and 45% were anemic. *Fasciola* eggs were detected in 9.4%, *Ascaris* in 13.4%, *Hymenolepsis nana* in 10.7%, *Trichiuris* in 1.7%, hookworm 1.4% and *Strongyloides* in 1.4%. *Giardia* was detected in 29% and *B. hominis* in 53%. Overall, 48.6% had at least one parasite, excluding *B. hominis*. Fascioliasis prevalence varied by community: 17.5% Huayllapata, 16.7% Piscohuata, 9% Huacaycancha 4.7% Chinchayhuasi, 3.8% Queunacancha, and 0% Ohuay (p=0.01). *Eosinophilia* (>500 eosinophils/µL) was present in 21% and also varied by community: 48.6% Ohuay, 31.6% Chinchayhuasi, 28% Huayllapata, 10% Huacaycancha, 8.9% Piscohuata, and 0% Queunacancha (p<0.01). Although, the overall prevalence of *Fasciola* in Huancarani district placed it as a meso-endemic area, the community level prevalence range from hypo to hyper-endemic areas within a very small geographic area. The high prevalence of eosinophilia suggests that some children were infected with undetected helminthes (e.g. acute phase of fascioliasis).

## 416

### ANTI-APOPTOTIC EFFECT OF *OPISTHORCHIS VIVERRINI*-THIOREDOXIN-1 (OV-TRX-1) - HUMAN APOPTOSIS SIGNAL REGULATING KINASE-1(ASK-1) IN CHOLANGIOCYTES

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*Opisthorchis viverrini* is a carcinogenic human liver fluke highly endemic in Thailand, Lao PDR, Cambodia and Vietnam. Infection is acquired by eating uncooked freshwater cyprinoid fish and can induce carcinoma of the bile duct (cholangiocarcinoma). Apoptosis/anti-apoptosis is one of the major mechanisms in the carcinogenesis of this cancer. Our study aimed to investigate apoptotic effects of *O. viverrini* thioredoxin-1 (OV-Trx-1) and Apoptosis signal-regulating kinase-1 (ASK-1) in human cholangiocyte (H69) using oxidative stress model. The recombinant Ov-Trx-1 protein was expressed in *E. coli*, purified on NI-NTA (6xHis tag) column and LPS removal by Triton-X114. H-69 cells were incubated with Ov-Trx-1 for 24, 48 and 72 h and 300 μM hydrogen peroxide was added to induce apoptosis. Harvested cells were measured for apoptosis by Annexin-Valexa488/PI using a Flow Cytometer. Immunolocalization of the Ov-Trx-1 in the cells was done by Immunofluorescence. mRNA expression of apoptosis and anti-apoptosis-related genes (i.e., BAX, ASK1, Caspase9, Caspase8, Caspase3, survivin, Mcl-1, JNK1/2, P38K) was performed by RT-PCR assay. Immunoprecipitation of cell lysates using His-Mag sepharose® bead was analyzed by immunoblotting with specific primary antibodies (anti-ASK-1, anti-His, anti-Ov-Trx-1, anti-Trx-1, anti-beta actin). Ov-Trx-1 showed anti-apoptotic activity in H-69 cells induced by H2O2. Ov-Trx-1 incubated cells showed decreasing mRNA expression of the apoptotic genes compared to controls. Immunolocalization revealed OV-Trx-1 was observed in the H69 cells by immunofluorescence. Immunoprecipitation of cell lysates showed that Ov-Trx-1 could bind to human ASK-1 by immunoblotting. The results suggest that OV-Trx-1 may play an important role in anti-apoptosis mechanism in pathogenesis of liver fluke induced cholangiocarcinoma.

## 417

### WAXING AND WANING PULMONARY CYSTS: A FLUKE?

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Global travel is associated with increasing incidence of helminthic infections in non-endemic regions. We report a case of patient with pulmonary paragonimiasis in Upstate New York who presented with recurrent hemoptysis. 20 year old healthy female college swimmer was referred for evaluation of intermittent episodes of hemoptysis for 1 year. Her symptoms began after a dive 3 meters above the pool resulting in a "belly flop". Complete blood count with differential was within normal limits. Initial chest x ray was normal. CT thorax showed 2 cystic lesions in the right lower lobe. The patient denied alcohol, cigarette or illicit drug use. Travel history was significant for a trip to Costa Rica and Bahamas 1 year prior. She continued to note 1-2 episodes every other week of quarter sized hemoptysis associated with shortness of breath. Bronchoscopy with bronchoalveolar lavage, brushings, and CT guided biopsy results were unremarkable. Cultures from the bronchial washings and the cytology were negative. Pulmonary Function Tests were normal. Workup for connective tissue disorders was negative. Further evaluation for pulmonary TB, mycoplasma, histoplasmosis and blastomycosis was negative. Subsequent CT scans showed interval resolution and redistribution of the cystic lesions. Paragonimus westermani antibody titers were ordered and found to be elevated at 1:32. The diagnosis of chronic pulmonary paragonimiasis was made and the patient responded well to praziquantel. Presentation of hemoptysis after diving with the finding of cysts on chest CT led to a consideration for traumatic pneumatocele. However, given the relatively mild chest injury at the time of the dive, cysts that resolved and recurred and the recent travel history, an infectious etiology was more likely. Although stool, sputum, and bronchial washings for ova and parasites were negative, *Paragonimus westermani* titers were elevated. Stool studies are insensitive and eosinophilia is much less common in chronic paragonimiasis. Serologic testing for anti-*Paragonimus* IgG however has a sensitivity of 100% and a specificity of 91%-100%. Paragonimus westermani also known as the lung fluke, is acquired through the ingestion of raw or undercooked crabs or crayfish and is the most common cause of hemoptysis worldwide. This clinical vignette underscores the importance of health care providers in the United States to recognize common worldwide infections.

## 418

### SOIL-TRANSMITTED DISEASES AND HUMAN FASCIOLIASIS WITH FEVER AND HIGH *EOSINOPHILIA* IN GEORGIA

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Soil-transmitted Helminthiasis and Fascioliasis are the most widespread parasitic diseases in Georgia. Wide geographic distribution makes these diseases as a major health issue in Georgia. Medical examinations to reveal Fascioliasis and helminthiasis cases were conducted in West Georgia during 2007-2011. For this pilot study 10 districts of Imereti region were selected. 1046 residents had high eosinophilia, digestion system complains and fever for several days in anamnesis. The main diagnostic test used for helminthiasis is an ovoscopic examination of stool by Kato-Katz method. Among 1046 individuals 313 (30%) were infected with different types of helminthiasis with 172 (50%) cases representing soil-transmitted diseases. Among those with soil-transmitted diseases 37% had Ascariasis and 12% had Trichuriasis. Human fascioliasis was present in 5% of study participants. The study has shown that historically widespread Ancylostomiasis (Hookworm), caused by Necator americanus and *Ancylostoma duodenale* were not identified among study participants. During epidemiologic investigation WHO experts recorded several species of lymnaedae - intermediate hosts of Fasciola. The study revealed that the prevalence of helminthiasis is high among residents of rural areas (37,5%) and suburbs (20%). The investigation showed that all favorable factors for the spread of soil-transmitted parasitic diseases is present in the Imereti Region and it's necessary to ensure that epidemiologic surveillance, preventive and treatment measures are adequately implemented. In addition, there is an urgent need for better monitoring and control of helminthiasis using new technologies.

## 419

### DEVELOPMENT OF COPRO-ANTIGEN DETECTION OF *OPISTHORCHIS VIVERRINI* USING RECOMBINANT CATHEPSIN F

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*Opisthorchis viverrini,* a food-borne trematode parasite as a major public health problem in Southeast Asia, particularly in Thailand, Laos, Cambodia and Vietnam. Chronic and repeated infection with *O. viverrini* is associated with the fatal cholangiocarcinoma, a rare cancer of the bile duct. Diagnosis of the infection is conventionally by stool examination. Research indicates that several parasite cysteine proteases have potential as diagnostic candidates. Cathepsin F is a major cysteine protease produced by eukaryotes including human liver flukes. We investigated whether *O. viverrini* Cathepsin F would be suitable for immunodiagnosis of human opisthorchiasis. Recombinant *O. viverrini* Cathepsin F (rOV-CF) was expressed in BL21 (DE3), purified by means of a His Trap column and gradually refolded overnight. rOV-CF was used for immunodiagnosis by Sandwich ELISA. Gaining capture antibody, two 18-week-old egg-laying Gallus domesticus chicken were immunized with 0.5 mg of purified rOv-CF mixed with equal amounts of adjuvant followed by 4 boosts. Eggs were collected and IgY extracted using the PEG 6000 precipitation method. Sandwich ELISA was performed by coating the plate overnight with 100 µl of carbonate-bicarbonate buffer containing 5 µg of purified IgY at 4ºC. The plate was blocked then sequentially incubated with a 2-fold serial dilution of *O. viverrini* somatic antigen and positive and negative samples of hamster feces. Rabbit anti-Ov-IgG from our previous study was used at a dilution of 1:500 (v/v). Peroxidase-conjugated goat anti-rabbit IgG at a dilution of 1:5000 (v/v) was used as the secondary antibody. The color was developed using HRP ELISA substrate and the absorbance was recorded. SDS-PAGE results showed a 40 kDa single band of mature rOv-CF and enzyme activity test verified it as an active cathepsin F. The Sandwich ELISA revealed that chicken IgY was able to capture the cathepsin F as low as 1 µg of *O. viverrini* antigen. Twenty three *O. viverrini* infected hamster feces, but not uninfected ones showed positive results. However, the background absorbance was still high in negative control. Further refinement is in progress. The Sandwich ELISA established in this study yet promising in *Opisthorchis* antigen detection in animal or human faecal samples.

## 420

### TRANSCRIPTOME ANALYSIS OF *PARAGONIMUS KELLICOTTI* MAY LEAD TO DEVELOPMENT OF IMPROVED SEROLOGICAL DIAGNOSTIC TESTS FOR PARAGONIMIASIS

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*Paragonimus* infections are widely distributed throughout Asia, Africa and the Americas and are among the most prevalent food-borne trematode infections. Following ingestion, parasites penetrate the intestine and make their way to the lung, causing symptoms that range from abdominal discomfort to severe lung disease. Safe and highly effective medications are readily available, but misdiagnosis often results in prolonged suffering and subjection to uncomfortable but ineffective treatment regimes tailored to unrelated illnesses (cancer, tuberculosis, etc). This is particularly common in North America where *P. kellicotti* is a relatively rare infection that physicians are not used to encountering. In most cases, specific IgG antibodies can be detected with the onset of pathological symptoms, often before parasite eggs can be detected in sputum, bronchoalveolar lavage, or stool. Characterization of the parasite antigens that stimulate this immune response would facilitate the design of improved diagnostic tools that could greatly improve patient outcomes. Unfortunately little information about the genome, transcriptome, and proteome of *Paragonimus* is available at this time. In order to address this need, we sequenced the transcriptome of developing adult *P. kellicotti*. Flukes were collected from experimentally infected gerbils 3-6 weeks post infection, and RNA was isolated and sequenced. A total of 120 million 100bp paired-end sequences were generated. High-quality read pairs were assembled into 246,149 consensus sequences. Despite obvious fragmentation, comparison with core eukaryotic genes suggests that approximately 79% of all *P. kellicotti* genes are represented in the initial transcriptome assembly. Translated transcripts will be used to facilitate characterization of peptides from mass spectroscopy analysis in order to identify parasite proteins recognized by the sera of infected patients. We will report on the progress of the improved transcriptome, the genome and the identification of immunodominant proteins that may lead to improved diagnostic tests for Paragonimiasis.

## 420A

### A GRANULIN GROWTH FACTOR SECRETED BY THE CARCINOGENIC LIVER FLUKE, *OPISTHORCHIS VIVERRINI*, AND ITS ROLE IN CARCINOGENESIS

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The human liver fluke, *Opisthorchis viverrini*, infects 9 million people throughout South-East-Asia and is a major cause of cholangiocarcinoma (bile duct liver cancer). In fact, as many as one-sixth of infected patients will develop liver cancer. The mechanisms by which the parasite causes cancer are multi-factorial, but one process is the secretion of mitogenic parasite proteins into the bile ducts, driving cell proliferation and creating a tumourigenic environment. Using proteomics and transcriptomics to characterise the *O. viverrini* secretome, we identified Ov-GRN-1, a homologue of the human growth factor, granulin. Previously we demonstrated potent growth factor activity (nanomolar) of recombinant Ov-GRN-1 on human biliary cells. Here we show that Ov-GRN-1 induces wound closure *in vitro* and results from *in vivo* wound healing experiments are ongoing and will be presented. Ov-GRN-1 stimulated angiogenesis (blood vessel formation) *in vivo*, a process critical for cancer development. Binding of recombinant Ov-GRN-1 to biliary epithelial cells induced dramatic changes in protein expression. Indeed, and in support of our hypothesis, many of the cellular proteins that were upregulated in response to Ov-GRN-1 were associated with cell growth and cancer, whereas the downregulated proteins were associated with tumour/proliferation suppression. Finally, silencing of Ov-grn-1 gene expression using RNA interference resulted in reduced mitogenicity of biliary cells by *O. viverrini* excretory/secretory products. Our novel findings contribute to the understanding of host-parasite interactions, and begin to address the mechanisms by which this parasite causes such a devastating form of cancer.

## 421

### INVESTIGATING THE ROLE OF CATHEPSIN B IN THE FREE-LIVING HELMINTH *SCHMIDTEA MEDITERRANEA* AS A MODEL FOR THE PARASITE *SCHISTOSOMA MANSONI*

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Schistosomiasis, a disease caused by the blood fluke genus *Schistosoma*, affects over 200 million people worldwide, especially in developing

countries. While not often fatal, chronic infection can damage internal organs and delay growth in children. It is known that proteolytic activity in *Schistosoma mansoni* is key to host invasion and worm survival. Characterizing the biological function of these proteases could lead potential drug targets. Unfortunately, direct assays like RNAi are not easily done in *S. mansoni,* making it difficult to determine the role played by specific proteases. However, RNAi can be used in *Schmidtea mediterranea*, a closely related free-living flatworm, which has been widely studied due to its ability to entirely regenerate and remodel its body tissues following injury. Although little is known about the proteome of *S. mediterranea,* its genome has been sequenced and is publicly available. Our aim is to identify *S. mansoni* protease homologs in *S. mediterranea* and determine biological function in both flatworms. After conducting an informatics-based survey of proteases in *S. mediterranea*, we are currently investigating the activity of cathepsin B, a cysteine protease in the papain family. This protease has been suggested as both a potential vaccine target and a serodiagnositc marker in *S. mansoni*, where it is localized to the gut and is involved in digestion of host albumin and hemoglobin. Preliminary data show a possible regeneration phenotype of cathepsin B knockdown in *S. mediterranea*, suggesting that cathepsin activity may be necessary for tissue remodeling. Western blotting has revealed a change in active cathepsin levels after a period of starvation with or without body amputation, another indication of involvement in regeneration and remodeling. It is also likely that cathepsin B performs a role in digestion, although more work is being done to confirm this. We are currently purifying cathepsin B from planaria lysates, as well as generating a specific antibody to determine its localization in *S. mediterranea*.

## 422

### ANTI-*SCHISTOSOMA MANSONI* RESPONSE IN MICROSCOPY NEGATIVE PARTICIPANTS

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The recommended strategy for Schistosomiasis morbidity control in Ghana relies on praziquantel chemotherapy. Though microscopy is a gold standard for diagnosis, it does not detect infection in endemic participants who are no longer shedding eggs because they are trapped in tissues. Schistosomiasis control cannot be separated from effective diagnosis, hence the need to screen sera for epidemiologic transmission markers to define exposure. This study determined anti-*Schistosoma* antibodies (IgM and IgG) to Soluble Egg Antigen (SEA) and Soluble Worm Antigen preparation (SWAP) in microscopy negative participants living in an endemic area. In a larger study, systemic random sampling was done to select 107 negative participants whose stool tested negative for *S. mansoni* ova and their sera screened with Enzyme Linked Immunosorbent Assays (ELISA) to detect anti-IgM and IgG antibodies. Eight microscopy positive sera were included to validate the ELISA test as well as compare SWAP and SEA responses to negative participants.Microsoft excel and SPSS 16 was used to organize data as well as check for statistical differences in microscopy and ELISA test values using chi$^2$ test at 95% confidence interval. For IgM, IgG and IgM/IgG sera showed 52.3%, 22.4% and 59.8% for SWAP and 31.0%, 46.0% and 53.27% for SEA respectively ( N= 107 for each test). Positive sera showed 50%, 88% and 88% for SEA and 62.5, 62.5% and 25% for SWAP IgM, IgG and IgM/IgG respectively, (N= 8 for each test). In effective disease control, serum antibody was useful in defining exposure, grouping acute and chronic infections in negative participants. Responsiveness of sera to SEA and SWAP could be affected by intensity and prevalence.

## 423

### SCHISTOSOMIASIS ELIMINATION: IDENTIFYING STRATEGIES AND PATHWAYS FORWARD

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Current WHO guidelines recommend a dual strategy for the control of schistosomiasis; (1) morbidity control adapted to the country context based upon regular mass drug administration (MDA) of praziquantel (PZQ) in high burden areas, and (2) a non-specific integrated control strategy (including the provision of safe water, adequate sanitation, and snail control) in areas where low endemnicity has been achieved and elimination may be feasible. In January 2012, the London Declaration on Neglected Tropical Diseases called for a coordinated push towards improved control globally, with focal elimination of schistosomiasis by 2020. However, current WHO and national governmental strategies are not aligned with the global goal of targeted elimination and are unlikely to achieve these benchmarks. To achieve focal elimination, the schistosomiasis community must rethink current strategic approaches. We sought to outline current WHO recommended schistosomiasis strategies and compare them to a proposed prevalence-specific elimination strategy. This prevalence-specific strategy is based on the understanding that more intensive MDA programs involving broader coverage and more frequent treatments can cure most infections and prevent reinfection. Moderate or high risk communities will require MDA of PZQ for 4 or more years and communities that attain low-prevalence and no longer require MDA therapy must utilize non-MDA based interventions to achieve complete transmission interruption. Specific snail control, sanitation, and diagnostic recommendations are designed according to the disease prevalence and risk level of a given community. Differences between WHO and the suggested new approaches to schistosomiasis elimination are highlighted and the potential disease reduction impact of both approaches is provided and compared. Specifically, the proposed strategy can be expected to increase successful cure rates, reduce community-level reinfection rates, and prolong longevity of transmission interruption relative to the current WHO strategy.

## 424

### SEROLOGICAL-PROTEOME OF THE PARASITE *SCHISTOSOMA MANSONI*: AN APPROACH FOR DIAGNOSTIC BIOMARKERS IDENTIFICATION

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Despite intensive efforts towards schistosomiasis control, the disease is still one of the most prevalent in the world. Improvements in the diagnostic would represent a step forward to the transmission control, being a suitable diagnostic assay essential for epidemiological surveys. Diagnosis procedures require continuous adaptation to the disease control stage and assays that are simple, inexpensive, sensitive, specific and able to distinguish active from prior infection have yet to be developed. Progress on post-genomic technologies resulted in a more rational approaches for new biomarkers discovery. A total of 47 different immunoreactive proteins were identified by our group using *Schistosoma mansoni* adult worm protein extracts probed with pooled sera of infected (INF), non-infected individuals from endemic area (NE) and of non-infected individuals from non-endemic area (NI), in a two-dimensional Western-blotting assay (2D-WB). Of these proteins, seven reacted exclusively to the INF sera pool, suggesting a possible use of this antigen panel to diagnostic purposes. Western-blotting (WB) with the *S. mansoni* recombinant protein Major Egg Antigen (rSmP40), one of the INF sera pool exclusively recognized antigen, showed a similar serum recognition profile to the native protein in

the 2D-WB. For further validation of the rSmP40 as diagnostic candidate, WB were conducted using the serum samples individually. Of the 12 INF serum samples, 8 (67%) recognized a protein band of approximately 40 KDa, corresponding to the rSmP40. None of the 8 NE (0%) and neither of the 7 NI (0%) serum samples were reactive to this same protein. Once the proteins which make up the panel of exclusively INF sera pool recognized antigens were identified simultaneously in a same 2D-WB, it is proposed that all of them might have the same potential as the rSmP40 for the development of a new diagnostic test. These antigens may be used as a diagnostic kit based on the detection of at least one of them, being capable to distinguish the clinical status of the schistosomiasis endemic area residents.

## 425

### IMMUNE RESPONSES TO MEASLES, MUMPS AND RUBELLA (MMR) VACCINATION AMONG SCHISTOSOMIASIS AND GEOHELMINTH INFECTED SCHOOLCHILDREN IN LEYTE, PHILIPPINES

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Schistosomiasis is a chronic and debilitating disease caused by helminths of the genus *Schistosoma*, affecting millions in the developing world including the Philippines. Helminths, including schistosomes, may suppress immune responses to evade attack, and we describe a study investigating immunoregulation of the measles, mumps and rubella (MMR) vaccine by schistosomiasis and helminth infections among schoolchildren in Leyte, Philippines. N=104 schoolchildren aged 7-16 from a highly endemic village for schistosomiasis consented and provided stool samples and blood pre- and post- MMR immunization. Kato Katz examination revealed that 38% of the cohort was infected with schistosomiasis, while 73%, 86%, and 20% were infected with *Ascaris*, *Trichuris*, and hookworm, respectively. Whole blood culture supernates of pre- and post-MMR immunization blood and stimulated with MMR antigens were assayed for 11 cytokines: Interleukin (IL)-1, IL-2, IL-6, IL-8, TNFα, IL-12, Interferon γ, IL-4, IL-5, IL-13 and IL-10. In multivariate regression analysis, schistosomiasis infection was associated with increased IL-1 (p=0.02) and IL-8 (p=0.05) levels in MMR-stimulated supernates collected at baseline. However, neither schistosomiasis nor geohelminth infections were associated with cytokine responses post-MMR immunization. Robust Th1 and pro-inflammatory cytokine response were observed at this timepoint, consistent with vaccination for viral antigens. Preliminary results of MMR-specific antibody responses by ELISA suggest that schistosomiasis or geohelminth infection were not associated with differences in post-vaccination antibody responses. Overall, these data suggest that schistosomiasis potentially enhances pro-inflammatory responses to bystander antigens (MMR), however MMR vaccine-induced antibody and cytokine responses may not be altered by schistosomiasis or geohelminth infection. Further data on neutralizing antibody responses by the measles plaque reduction neutralization test will be presented.

## 426

### BIOCHEMICAL CHARACTERIZATION OF SERINE PROTEASE INHIBITORS FROM *SCHISTOSOMA JAPONICUM* AS NOVEL TARGETS FOR PUBLIC HEALTH INTERVENTIONS

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Schistosomiasis remains a major global public health problem, but despite significant progress in the control of this disease, clear limitations necessitate the development of an effective anti-schistosomal vaccine. Serine protease inhibitors (serpins) are a superfamily of proteins involved in many important biological processes such as blood coagulation, fibrinolysis and inflammation. These inhibitors are known to play central roles in host immune modulation and/or evasion by pathogens. Furthermore it has been suggested that serpins may have evolved specifically in limiting the host immune activation by mediating the inhibition of host immunomodulatory signals. We hypothesise that serpins provide similar functions for schistosomes and their disruption by an effective host immune response by vaccination provides an opportunity to eliminate and control these parasites. Therefore, the aim of this study was to biochemically characterise novel serpins from *Schistosoma japonicum* and investigate the potential of these proteins as suitable anti-schistosomal vaccine candidates. Gene expression results from data mining of previously published microarray findings of our group and subsequent confirmation with quantitative PCR showed that two *S. japonicum* serpins termed *SjB6* and *SjB10* are differentially expressed in different life cycle stages of the parasite. The highest relative gene expression was observed in the egg and cercarial stages for *SjB6* and *SjB10* respectively indicating possible pathological and/or immunological relevance as well as a possible role for SjB10 in cercarial penetration. Western blot analysis confirmed the expression of the native proteins in the adult worms. Recombinant proteins produced were tested for their inhibitory activity against a panel of serine proteases. SjB10 was shown to be biochemically active against pancreatic elastase and chymotrypsin while SjB6 showed no activity against any of the proteases tested. Work is now ongoing for the immunolocalisation of the native proteins using polyclonal antibodies raised in rabbit as well as evaluating their possible anti-schistosomal vaccine efficacies.

## 427

### T LYMPHOCYTE PROFILE AND ACTIVATION STATUS IN SCHISTOSOMIASIS PATIENTS WITH LIVER FIBROSIS

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Schistosomiasis takes the second place in clinical and epidemiological magnitude among parasitic diseases in the world. The host immune response is crucial for the protection and pathology. Our hypothesis is that an impaired T regulatory response plays a role in the development of periportal fibrosis in human schistosomiasis. In this study we evaluate the phenotype and the degree of T cell activation in schistosomiasis patients with periportal fibrosis. We included 37 subjects living in the village of Agua Preta, Bahia, Brazil. Periportal fibrosis was determined by ultrasound. Peripheral blood mononuclear cells were obtained by the Ficcol-Hypaque gradient and the expression of the surface markers CD28, CD69, CD25 and CTLA-4 on TCD4+ and TCD8+ cells were determined by flow cytometry. The frequency of TCD4+ cells was similar in patients with different degrees of periportal fibrosis, however, individuals with moderate to severe fibrosis showed a lower frequency of TCD8+ cells when compared to patients without fibrosis or incipient fibrosis. The frequency of TCD4+CD28+ cells was higher in patients with moderate to severe fibrosis (mean = 95.7%) when compared to those with incipient fibrosis (62%). The frequency of TCD4+ and TCD8+ cells expressing CD69 did not differ between groups. The frequency of CD4+CD25Neg cells was higher in patients with moderate to severe fibrosis, compared to the other groups, while the frequency of CD4+CD25High cells was lower in patients with moderate to severe fibrosis (1.0%) compared to those without fibrosis (1.8%) or with incipient fibrosis (1.75%; $p<0.05$). The frequency of TCD4+CTLA-4+ cells in individuals with moderate to severe fibrosis was also lower (0.41%) than in patients without fibrosis (0.8%) or with incipient fibrosis (1.6%; $p<0.05$). The low frequency of T cells with regulatory profile in schistosomiasis patients with periportal fibrosis may be associated to the high frequency of activated T cells and therefore leading to liver pathology in these patients.

## 428

### GENETIC AND PHARMACOLOGICAL ANALYSIS OF TRANSIENT RECEPTOR POTENTIAL (TRP) CATION CHANNELS IN *SCHISTOSOMA MANSONI*

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Though schistosomiasis affects hundreds of millions worldwide, only a single antischistosomal drug (praziquantel) is available in most parts of the world, a potentially dangerous situation. Reports of praziquantel resistance lend particular urgency to the need for new therapeutics. Ion channels allow ions to flow by diffusion down the electrochemical gradient established across cell membranes, and are essential to normal functioning of the neuromusculature and other tissues. They are validated targets for drugs and toxins in a number of systems, and, indeed, the majority of current anthelmintics act on ion channels. However, only a very few of the ion channels expressed by schistosomes and other parasites have been analyzed for their potential as targets for new drugs. One set of channels with promise for exploitation is the transient receptor potential (TRP) channel superfamily. TRP channels are a highly diverse superfamily of cation channels that share the common feature of mediating transduction of sensory signals. TRP channels also appear to play roles in modulating immune responses and lifespan, and are being intensively investigated as drug targets for a variety of human conditions ranging from chronic pain to cancer. To date, however, the functions and pharmacological sensitivities of schistosome TRP channels have not been determined. We are using pharmacological, genetic, and molecular biological tools to better understand the roles these channels play in *Schistosoma mansoni* physiology and survival. For example, we find that agents that selectively activate or antagonize mammalian TRP channels have dramatic effects on motility of *S. mansoni* adults and schistosomules. We are also using RNA interference to suppress expression of these channels and help further establish their role and potential for targeting. These studies, in parallel with heterologous expression studies, should provide important information about schistosome sensory physiology as well as novel candidate drug targets.

## 429

### ASSOCIATION BETWEEN CHANGES IN SOCIOECONOMIC STATUS, WATER CONTACT AND *SCHISTOSOMA MANSONI* INFECTION IN AN ENDEMIC AREA IN MINAS GERAIS STATE, BRAZIL

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We evaluated changes in *Schistosoma mansoni* prevalence, socioeconomic status and water contact behavior for a cohort of 357 individuals between 2001 and 2009 in an endemic rural area Minas Gerais State, Brazil. Parasitological surveys using three stool samples per survey and the Kato-Katz method were carried out in 2001 and 2009 and all positive individuals were treated with praziquantel. Information on socioeconomic status (SES) and frequency of water contact was collected for each individual during household surveys using questionnaires. We used several binary variables indicating changes in household possessions and motorized vehicles between 2001 and 2009 for each individual. A generalized linear model was used to assess the association between demographic variables, SES and water contact with *S. mansoni* prevalence in both models (infection and reinfection). We also used a Bayesian method of variable selection to identify the most significant factors associated with prevalence. *S. mansoni* prevalence before treatment (2001) was 59.0%, with a geometric mean egg count (epg) of 61.05. In 2009, prevalence was 26.8% and intensity of infection had significantly declined to 8.78 epg. There was a slight decrease in the number of individuals who had unsafe contacts between 2001(75.3%) and 2009(70.8%). The Bayesian selection model revealed that changes in type of water contact from safe to unsafe and persisting poverty (individuals who had not acquired durable goods or improved their houses between 2001 and 2009 were jointly relevant in describing the risk of *S. mansoni* infection during the 8-year study period. Based on these results we conclude that both SES and water contact factors can be predictive of changes in *S. mansoni* prevalence in longitudinal studies using Bayesian analysis.

## 430

### PRESCHOOL AND SCHOOL CHILDREN MALNUTRION AND SCHISTOSOMIASIS IN A VILLAGE FROM BENGO PROVINCE (ANGOLA): PRELIMINARY RESULTS FROM AN INTERVENTION STUDY

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Malnutrition and anemia are two of the potential consequences of Schistosomiasis, soil transmitted helminths and malaria. Those conditions coexist as endemics and potential factors causing several clinical consequences in children and compromising not only their growth and development but also their academic performance levels. The prevention and control of this disease involve the preventive administration of Praziquantel, and Albendazol, improvements in fresh water supplies, healthcare and education. This work presents the first results of a broader project aiming to contrast the results of the effectiveness of a school based programs and those of a community based programs as well as between pre-school age children and school age children, in Bengo province, Angola. An intervention study in a village from Angola, of 95 preschool children (2-5 year olds), 115 school-aged children (6-15 year olds) and 185 adults (> 15 year olds) was conducted to evaluate malnutrition, anemia, malaria, schistosomiasis and geohelminths and treat all intervenient with Praziquantel and Albendazol. Results: Malnutrition was common among children. In preschool children 13.4% were under-weight and 55.2% stunting. In school children 16.6% were under weight and 22.7 stunting. Anemia (<11.0 mg /dL) was found in 65% of children. Urinary schistosomiases prevalence reached 67% of preschool children and 82 % of school-aged children. Geohelminth infections were common, affecting 32% of preschool children and 58% of school-aged children. In conclusion, we report the first results of the intervention study and the results obtained justify the implementation of the interventions for the control of these diseases and morbidities, namely malnutrition and anemia. During the present year the effectiveness of the intervention and a comparison with a school intervention in a different village will be conducted.

## 431

### A CASE-CONTROL STUDY OF NOROVIRUS-INDUCED ACUTE DIARRHEA AMONG ARMY RECRUITS IN PERU

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Norovirus is the number one cause of acute gastroenteritis worldwide and a leading cause of diarrhea deaths in children under five years old. However, few data are available on the incidence of norovirus in adults in developing countries. We conducted active surveillance for acute diarrheal illness among army recruits at the Vargas Guerra Army Base in Iquitos, Peru, from October 2005 through July 2011. To determine the frequency

of norovirus-induced diarrhea in this population, we performed a nested case-control study on 200 randomly selected cases with acute diarrhea and 200 randomly selected asymptomatic controls. Stools were tested for norovirus by RT-PCR; bacteria using culture and RT-PCR for *Escherichia coli*; and parasites using direct microscopy. Uni- and multivariate analyses were performed using Stata statistical software. Norovirus was present in 14.1% of cases and 8.0% of controls, with genogroup GII in 71%, GI in 25%, and mixed GI/GII in 4%. Norovirus-bacterial co-infection was present in 30%, including Shigella flexneri (15.4%) and enterotoxigenic *Escherichia coli* (15.4%). Norovirus-parasite coinfection was noted in 62% of cases, including *Trichuris trichiura* (27%), *Giardia* species (27%), *Ascaris lumbricoides* (23%), and *Uncinaria stenocephala* (8%). After controlling for co-infections, any norovirus infection and norovirus GII infection were associated with diarrhea, with adjusted odds ratios (AOR) of 2.8 (95% CI: 1.3-6.0, P<0.01) and 3.6 (95% CI: 1.4-9.2, P<0.01), respectively. Norovirus GI infection was not associated with diarrhea (AOR: 1.4, 95% CI: 0.4-4.3, P=0.61). Although norovirus was a frequent cause of acute diarrhea in this population, its frequent presence in controls suggests that additional factors beyond simple presence/absence of infection play roles in the development of disease. These may include size of the inoculum, co-infections, previous infection and adaptive immunity, and genetic predisposition. Further investigation of the possible role of these co-factors is warranted to fully understand the epidemiology of norovirus disease.

## 432

### THE STATUS AND CHALLENGES FOR THE CONTROL OF RABIES IN NEPAL

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A study was conducted in Nepal to know the status of rabies. Five years data (from 2005 to 2009) on human rabies information recorded at Epidemiology and Disease Control Division (EDCD) under the Department of Health Service and animal rabies information recorded at Veterinary Epidemiology Centre (VEC) as well as Central Veterinary Laboratory (CVL) under the Department of Livestock services were analyzed to know the status of Rabies in Nepal. There were 411 outbreaks and 750 animal deaths in 45 (out of 75) districts of country from 2005 to 2009. A total number of 48,703 animals were given post exposure treatment after bitten by suspected rabid animals. Rabies has been reported in dogs, cattle, buffaloes, sheep, goat, pigs and horses. Over the 5 years, 112 dogs, 19 buffaloes, 14 cattle, 11 goats, 2 cats, 2 mongooses, 1 rabbit, 1 mouse, 1 bat and 1 swine brain samples were submitted at CVL for laboratory confirmation. In total 164 clinical samples were tested out of which 113 (68.9%) were positive for rabies. Rabies has been confirmed in 59.82% (67/112) dog, 84.21% (16/19) buffalo, 78.57 (11/14) cattle, 72.72 (8/11) goat, 50% (1/2) mongoose and 100% (1/1) mice samples. The total numbers of rabid human (hydrophobia) cases admitted to hospital from 2005 to 2009 were 163 however all of these cases were diagnosed on the basis of clinical symptoms and history of dog bite. None of the human cases were confirmed on the basis of laboratory tests. In total 120,398 persons had received post exposure treatment in this period from 48 hospitals at free of cost after bitten by suspected rabid animals.

## 434

### THE ROLE OF IKKβ IN VEEV REPLICATION

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Venezuelan equine encephalitis virus (VEEV) belongs to the genus Alphavirus, family Togaviridae. Currently no antivirals or therapeutics are available to treat VEEV infections. Our preliminary studies with small molecule inhibitors had indicated that the NFkB cascade, more specifically the IKK component, may play an important role in VEEV infection of human cells. We aimed to characterize the interaction of the host IKK complex with the virus and the consequence to viral multiplication. Initial findings indicated that at early time points of VEEV infection the NF-κB complex was activated when compared to UV-infected samples (as inferred by phosphorylation of p65 and IkBa). The upstream event contributing to phosphorylation of p65 is the activation of the IKK complex. Our previous studies with a different virus (Rift valley fever virus) had indicated that the IKKβ component underwent macromolecular reorganizatio to form a novel form of the complex that was unique only to infected cells. This prompted us to investigate if the IKK complex undergoes a comparable macromolecular re-organization in VEEV infection. Column fractionated mock and VEEV infected cell extracts indicated a macromolecular re-organization of IKKβ in VEEV infected cells, which resulted in formation of lower molecular weight complexes. Well-documented inhibitors of the IKK complex, BAY-11-7082, BAY-11-7085 and IKK2 compound IV, were employed to determine the effects of this inhibition on infectious viral titers. A decrease in infectious viral particles and viral RNA copies was observed with inhibitor treatment in VEEV infection. The efficacy of these inhibitors was also tested on the wild type strain of VEEV (TrD) in U87MGs and neuronal cells where are potent inhibition was observed. IKKβ over-expression studies increased TC-83 replication. In contrast, IKKβ-/- knockout studies demonstrated a reversed phenotype, where TC-83 replication was inhibited. Finally, *in vivo* studies demonstrated that inhibitor treatment of TC-83 infected mice increased in their mean survival time. Thus far our studies have revealed that the host IKKb protein may be critically involved in VEEV multiplication. Ongoing proteomic studies are aimed at determining the mechanism behind the alteration of the IKKb complex during VEEV infection.

## 435

### LASSA FEVER OUTBREAK INVOLVING HEALTH CARE WORKERS IN TARABA STATE, NIGERIA; MARCH 2012

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Lassa fever is an acute, highly infectious viral haemorrhagic illness caused by Lassa fever virus - a single stranded, RNA virus belonging to the virus family Arenaviridae. The reservoir is Mastomys natalensis. The disease is endemic in West African sub region causing 300,000-500,000 infections annually, with about 500 deaths. In March, 2012, we investigated a reported outbreak of Lassa fever in Taraba State, Nigeria to confirm the outbreak, determine its extent, characterize the outbreak, institute public health actions and make appropriate recommendations. We reviewed hospital records and used IDSR standard case definition for Lassa fever to identify and line-list cases. A suspected case was defined as "any person with severe febrile illness not responsive to the usual causes of fever in the area with or without sore-throat and at least one of the following: bloody stools, vomiting blood, bleeding into the skin and unexplained bleeding from the nose, vagina or eyes". A standardized line-listing form was developed to capture socio-demographic and clinical information of the cases. Various exposure factors including age, gender, occupation and contact history were examined. A total of 35 cases were recorded. Nine of 35 cases were laboratory confirmed (25.7%). Altogether, 14 deaths were recorded giving a case fatality rate of 40%. Majority of the cases belonged to the age group 25-34 years (40%) with females constituting 51%. Most of the cases were healthcare workers (22.9%). The commonest presenting features were fever (85.7%), cough (28.6%), bleeding from orifices or into skin (25.7%) and headache (20%). The State's Epidemic Management Committee was found to be non-functional resulting in uncoordinated response to the outbreak. In addition, many exposure factors to Lassa fever such as over-crowding, drying of food items along high ways and bush burning were identified and there was low index of suspicion of Lassa fever among health care workers. There was a confirmed outbreak of Lassa fever in Taraba State mostly affecting healthcare workers. Community sensitization and sensitization of health care workers in Taraba

State on Lassa fever were carried out. It was recommended that the State should reactivate its moribund Emergency Management Committee, surveillance of Lassa fever should be strengthened and Public/Health care workers sensitization activities should be scaled up.

## 436

### UNDERSTANDING THE DYNAMICS OF CIRCULATION, REASSORTMENT AND TRANSMISSION OF NGARI AND BUNYAMWERA VIRUSES IN NORTHERN KENYA

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Although genetic drift/reassortment seems to occur frequently in the Bunyaviridae family, the epidemiological consequences of these evolutionary events are poorly understood. Our objective was to understand the dynamics of circulation, reassortment and transmission of Bunyamwera and Ngari viruses in Kenya. We identified isolates of both viruses which were relatively conserved regardless of the species or region of isolation with the later clustering closest with the Ngari strain associated with the 1997-1998 hemorrhagic fever outbreaks in East Africa. *Anopheles* gambie was a more competent vector for Ngari than Bunyamwera virus possibly due to genetic reassortment. This has major implication in light of continued animal trade and travel especially into malaria endemic regions where *Anopheles gambiae* is more prevalent. Our results underscore the need to continually monitor emergent arboviral genotypes circulating within particular regions as well as vectors mediating these transmissions in order to preempt and prevent their adverse effects.

## 437

### HEPATITIS E VIRUS INFECTION AT THE HUMAN ANIMAL INTERFACE, LAGOS NIGERIA

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Hepatitis E virus (HEV) is a leading cause of acute/chronic liver failure with higher severity in pregnant women. About one million human deaths are annually recorded globally due to viral hepatitis of which HEV contributes significantly. Pigs are considered natural reservoir host of HEV especially in intensive farms with close human-animal interaction coupled with poor sanitary practices. The zoonotic risk of HEV among occupationally exposed individuals in developing countries is of public health concern. However, data on disease burden for planning intervention in sub-Saharan Africa is lacking. This study describes the seroprevalence, of HEV at the human-animal interface in a pig estate in south western Nigeria. Cross sectional sampling of blood from randomly selected pigs and pig handlers of a multi-complex pig farm in Lagos, south western Nigeria was carried out. Seventy three human and 212 swine sera were collected. Sera were tested for anti-HEV IgG and IgM antibodies by two step double antigen sandwich ELISA using Hep.EV. According to manufacturer's protocol, two hundred and twelve (97%,) and 13 (17.8%) of swine and human sera were positive for HEV total antibodies respectively while 3 (1.4%) and 1 (1.3%) of swine and human sera were positive for HEV IgM respectively. 70% of pig handlers tested were women most of whom are within the reproductory age and one was pregnant. The high seropositivity observed and evidence of recent infection in pigs is a cause for concern. This is because there is a high risk of transmission of the virus to human contacts in a farm where over 80% of farms operate under poor biosecurity and waste management. This study provides information on HEV disease burden in pigs and pig handlers in the study area and emphasizes the zoonotic risk of HEV. In controlling HEV in this tropical region therefore, improvement in biosecurity practices including sanitation and proper waste disposal is strongly advocated. Further study on the genomic diversity of circulating HEV strains and the risk of feco-oral trasmission is recommended.

## 438

### EFFECT OF ZINC SUPPLEMENTATION ON RESPONSE TO ORAL POLIO VACCINE IN INFANTS IN PAKISTAN: A RANDOMIZED CONTROLLED TRIAL

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Polio eradication remains a challenge in Pakistan and causes for the failure to eradicate polio are complex. Undernutrition and micronutrient deficiencies, especially zinc deficiency, have been identified as major public health problems in Pakistan and could potentially affect response to enteric vaccines. This study was undertaken to assess the impact of zinc supplementation among infants on immune response to oral polio vaccine (OPV). A double-blind, randomized placebo controlled trial was conducted in infants aged 0-14 days. Subjects were assigned to either treatment group receiving 10 mg zinc supplementation daily for 18 weeks or control group. Both groups received standard OPV doses at birth, at 6 weeks, 10 weeks and 14 weeks. Data was collected on demographic information and blood samples were collected to determine the antibody response to OPV and for micronutrient analysis. The prevalence of poliovirus seropositivity and seroconversion was examined using mixed effects logistic regression. Overall, 404 subjects were recruited; of which 303 were included in the crude analysis. At recruitment, seropositivity was already high for poliovirus (PV) serotype 1 (zinc: 89.7%; control: 90.4%) and PV2 (89.7%; 93.6%), with lower estimates for PV3 (69.9%; 65.6%). The proportion of seropositive subjects for PV3 at week 6 was higher for those in the zinc group [53.4 (45.3, 61.6)] than in the control [42.7 (34.9, 50.5)], albeit not significant (p=0.061). This trend was not observed at week 18. There were no differences between groups for PV1 or PV2 at either week 6 or week 18. In the multivariate logistic regression model, no association was found between seropositivity or seroconversion and zinc supplementation for either PV1, PV2, or PV3. In conclusion, zinc supplementation in Pakistani infants from two weeks of age was not associated with a significant impact on seroconversion to OPV.

## 439

### MUTATIONS PRESENT IN WESTERN EQUINE ENCEPHALITIS VIRUS POSSIBLY ACCOUNT FOR REDUCTION IN HUMAN INCIDENCE

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Western equine encephalitis virus (WEEV) has caused epidemics that have resulted in the deaths of thousands of humans and equids over the past century. Interestingly, WEEV infection has decreased precipitously over the past fifty years, with the last documented clinical case observed in 1998. Depite this decline, the virus has still been found to be cirulating in nature until 2008 as evidenced by virus positive mosquito pools. The purpose of this study is to discover any mutaitons in WEEV that could account for this observed reduction in human incidence. To accomplish this, a robust collection of WEEV viruses were collected and whole genomes were sequenced. In order to determine what mutations define specific lineages, maximum parsimony, neighbor-joining, maximum likelihood, and Bayesian phylogenies were generated and amino acid mutations were traced using these phylogenetic trees. To elucidate possible changes in genetic diversity such as population bottlenecks or logistic population growth, we used Bayesian skyline reconstructions implemented in BEAST. Additionally, we estimated evolutionary rates and dates of divergence for the entire phylogeny and individual lineages. Several mutations were identified and their inferred dates of divergence appears to coincide with WEEV's observed reduction in human incidence. These mutations also define important lineages in the phylogeny. They are located in nsP3, nsP4, capsid, E1, and E2 genes. These results supply compelling evidence that WEEV's evolution over the past half century has resulted in a reduction of human virulence. These mutations will now be characterized and tested in order to determine if they do impart a phenotypic effect.

## 440

### PERFORMANCE OF RISK EXPOSURE SCREENING QUESTIONS TO IDENTIFY NIPAH CASES ON ADMISSION IN SURVEILLANCE HOSPITALS IN BANGLADESH

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Drinking raw date palm sap and contact with Nipah case-patients are major pathways of transmission for Nipah encephalitis in Bangladesh. Ongoing hospital based meningo-encephalitis (ME) surveillance identifies Nipah cases through serological diagnosis; however, results often take weeks to obtain. Earlier detection of Nipah cases could improve our ability to prevent secondary transmission from patients to caregivers and healthcare workers through infection control. The objective of the study was to assess the performance of using reported risk exposures among ME cases in surveillance hospitals to detect Nipah cases on admission. From December 2012 to March 2013, study physicians in three Nipah surveillance hospitals assessed all ME cases for known risk exposures for Nipah infection by asking them or their caregivers if they had consumed raw or fermented date palm sap or had contact with any another ME case-patient in the month before onset of illness. We calculated the sensitivity, specificity, and positive predictive value (PPV) of these screening questions to identify Nipah cases by comparing them to results from IgM serology tests. We also calculated these performance measures for cases occurring during the peak Nipah incidence months in Bangladesh of January and February. From December 2012 to March 2013, we identified 360 ME cases and 328 (91%) had serum tested for anti-Nipah IgM. 19 (6%) cases had detectable IgM antibody against Nipah virus and 14 of these reported ≥1 known risk exposure, for a sensitivity of 74%. Among 309 ME cases who had no detectable IgM antibody, 264 had no known risk exposures, for a specificity of 85%. Among all ME cases, 59 (18%) had ≥1 known risk exposure, for a PPV of 24%. Among ME cases enrolled during January-February 2013, sensitivity was 93%, specificity was 83%, and PPV was 38%. In conclusion, screening for risk exposures on admission demonstrated high sensitivity and specificity for detecting Nipah cases, particularly during peak incidence months. On admission, screening criteria should be used to identify cases for whom more stringent infection control measures are necessary. Because the PPV of the screening questions was low, development of a rapid test kit for Nipah diagnosis would assist case detection. Considering limited diagnostic facility, low cost countrywide expanded surveillance focusing high risk exposures using screening criteria may identify more Nipah cases.

## 441

### COMPLETE GENOMES OF INFLUENZA A SEASONAL H3N2, 2009 PANDEMIC H1N1 AND INFLUENZA B DETECTIONS BY NEXT GENERATION SEQUENCING

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Next generation sequencing (NGS) Illumina, MiSeq Platform was utilized to investigate the complete genome of six influenza virus isolates, which were positively identified using the CDC polymerase chain reaction (PCR) assay. The DNA library obtained from each virus was mixed and all were sequenced simultaneously. Total information of 2.6 Gbases was obtained from a 455±14 K/mm$^2$ density with 96.76% (8,571,655 / 8,950,724 clusters) of the clusters passing quality control (QC) filters. Approximately 93.7% of all sequences from Read1 and 83.5% from Read2 contained high quality sequences that were ≥Q30, a base calling QC score standard. The average error rate from base calling at the 100$^{th}$ cycle was measured as 0.20%±0.03% for both reads. Total six alignments analysis identified three Influenza A seasonal H3N2 strains (A/Brisbane/11/2010), one 2009 pandemic H1N1 (A/California/07/2009) strain and two Influenza B (B/Wisconsin/01/2010) strains. The nearly entire genomes of all six virus isolates yielded equal or greater than 600-fold sequence coverage depth. MiSeq Platform efficiently identified DNA library mixtures of Influenza A seasonal H3N2, 2009 pandemic H1N1 and Influenza B.

## 442

### AN EPIDEMIC OF CHIKUNGUNYA IN NORTHWESTERN BANGLADESH IN 2011

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In November 2011, the primary healthcare (PHC) manager of Shibganj subdistrict of Chapainababganj District in northwest Bangladesh reported a cluster of patients with fever and joint pain or rash. We investigated the outbreak to identify the etiology, characterize the illness and estimate the attack rate. We defined a suspect case as a resident of Shibganj with fever, joint pain and/or rash with onset from September 1–December 31, 2011. To estimate attack rate, we conducted a household-based syndromic survey in 16 clusters using multistage cluster sampling. Trained field workers visited 80 households nearest to the local PHC clinic in each cluster to identify suspect cases. We invited one suspect case per household from the first 30 households per cluster to complete a structured questionnaire and provide a blood sample. We tested serum samples for immunoglobulin M (IgM) antibodies to Chikungunya virus (CHIKV) by capture enzyme-linked immunosorbent assay. Because of the time-lag between illness onset and laboratory investigations and the limited duration of IgM antibodies in serum, we used the number of suspect cases to estimate the attack rate. We surveyed 5,972 people from 1,283 households. Among these, 30% (1,769) met the suspect case definition; the median age was 28 (Interquartile range [IQR]: 15–42) years and 48% (856/1,769) were males. Among 480 suspect cases invited to participate in the sero-survey, 377 (79%) completed questionnaires; median age was 40 years and 64% (241/377) were females. 77% (377/480) provided a blood sample and 74% (278/377) had evidence of IgM antibodies to CHIKV. The outbreak lasted >3 months and peaked in early November. Predominant symptoms included fever (100%), joint pain (88%), debilitating weakness (55%), myalgias (44%), itching (33%) and rash (31%). The median duration of joint pain was 15 (IQR: 6-19) days and 7 (IQR: 5-13) days for debilitating weakness. 64% (239/377) of the suspect cases surveyed sought care from local PHC clinics. The 30% attack rate and the predominance of adult cases suggest that Shibganj's residents had little previous immunity to CHIKV. This was the second recognized epidemic in Chapainababganj, following a smaller outbreak in 2008, and underscores the continued risk of CHIKV epidemics in Bangladesh. Averting CHIKV in Bangladesh requires improved PHC-based surveillance to enhance outbreak detection, define transmission risk and allow early public health interventions.

## 443

### EVALUATION OF HUMAN INFLUENZA VIRUS ISOLATION USING MDCK CELL CULTURE DURING SURVEILLANCE IN ASIA

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Laboratory influenza surveillance is critical to monitor influenza activity and to provide the epidemiological data needed for emerging pandemic preparedness and vaccine development. In addition, sample collection followed by viral propagation is required for more complete characterization. Since 2008, USAMC-AFRIMS has conducted influenza surveillance in Bhutan, Nepal, Philippines and Thailand. Respiratory samples are routinely identified and characterized by real-time RT-PCR (rRT-PCR), in which influenza viruses are further sub-typed. Virus isolation is done for both influenza rRT-PCR positive and negative samples using Madin-Darby canine kidney (MDCK) cell culture. From 2008 to 2012, 5,667 samples were randomly selected for virus isolation. While 49% of all respiratory samples tested by rRT-PCR in that group were positive for influenza virus (2,803 samples of 5,667), only in 54% (1,508 samples of 2,803) were we able to successfully isolate and identify the virus by immunofluorescence assay (IFA). The isolation rate varied among the different subtypes. Influenza A H1N1, A H3N2, A 2009 H1N1, and B were successfully isolated in 77, 62, 34, and 64% of the rRT-PCR positive specimens, respectively. The rate of successful influenza virus isolation strongly correlated with the rRT-PCR results. Respiratory samples with high viral content with low cycle -to-threshold value ($Ct \leq 20$), moderate viral content ($20 < Ct < 30$), and low viral content ($Ct \geq 30$) were successfully isolated at the rates of 84, 51 and 6%, respectively. The isolation rate varied from country to country, with samples collected from Bhutan showing the lowest rate (17%). The low isolation rate, as compared to rRT-PCR results, may be attributable to viral viability loss during collection and transport (highlighting the importance of handling and cold-chain monitoring) but also to a less than adequate cell substrate. These analyses provide useful information to consider when designing future influenza surveillance studies.

## 444

### MOLECULAR CHARACTERIZATION OF ANTIVIRAL SUSCEPTIBILITY OF INFLUENZA A ISOLATES OBTAINED IN KENYA FROM 2008 TO 2011

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Presently, there are two main classes of antivirals in use which function by inhibiting specific steps within the virus replication cycle: M2 inhibitors block the uncoating of the virus through acidification of the interior of the virion. In neuraminidase inhibition, inhibitor molecules mimic NA's natural substrate and bind to the active site, preventing NA from cleaving host cell receptors and releasing new virus. The study characterized antiviral susceptibility of the 2008-2011 influenza A strains using known molecular markers in neuraminidase (NA) protein. In the 2008-2009, 2009-2010 and 2010-2011 influenza seasons, a total of 836 viruses were isolated. 344 (41%) were influenza A/H3N2, 144 (17%) seasonal influenza A/H1N1 and 348 (42%) belonged to the pandemic influenza A/ H1N1 strain. A total of 108 (13%) isolates were analyzed for susceptibility to NA inhibitors. In the year 2008, 33 influenza A/H3N2 and 11 seasonal influenza A/H1N1 were included in the genotypic characterization assay for neuraminidase inhibitor resistant mutations. Sequence assembly and alignment revealed absences of molecular markers of neuraminidase inhibitor drug resistance (Y275) in influenza A/H3N2. 64% (7) of the 2008 seasonal influenza A/ H1N1 isolates had resistant marker H275Y. 4 (36%) of the seasonal A/ H1N1 isolates, lacked the drug resistant marker depicting sensitivity to the class of drugs. Genetic analysis of the 48 pandemic influenza A/ H1N1 strains in 2009 showed that all were sensitive to oseltamivir through possession of histidine at position 274 of the neuraminidase protein sequence. The same pattern was duplicated in 2 of the pandemic influenza A/ H1N1 isolates analyzed in the year 2010. All the 2011, 14 isolates belonging to influenza A/H3N2 subtype lacked the H275Y substitution in the neuraminidase protein. Genotypic data obtained in this study demonstrate antiviral resistance in seasonal influenza A/H1N1 viruses isolated in Kenya in 2008-2009 through possession of H275Y (N1 numbering) marker in the neuraminidase protein.

## 445

### INFLUENZA A VIRUS IN SWINE IN PERU

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Influenza viruses belong to the Orthomyxoviridae family and are divided into 3 types (A, B and C). Influenza A viruses are further classified into 17 HA and 9 NA subtypes that can infect a variety of hosts, including migratory waterfowl, resident birds, horses, swine, dogs, sea mammals, guinea pigs, bats and humans. Although different strains have evolved with each species, cross transmission of some influenza viruses between species is possible. Swine play a particularly important role as "mixing vessels" because they possess receptors recognizing both human and avian influenza A viruses. We sought to determine the prevalence of influenza A virus in swine raised in the Department of Lima and surrounding areas of the central coast of Peru. Surveillance was conducted at a central slaughterhouse in Lima. Upon arrival at the facility, animals were inspected for health status and kept in groups according to the seller for a maximum of 14 hours. Blood and nasal and tracheal swab specimens were collected at the time of slaughter. Serum was tested for IgG antibody to influenza A virus by ELISA (IDEXX Laboratories, Maine, USA) and the swab samples by rRT-PCR using the CDC Flu A assay to detect universal influenza A virus. PCR positive samples were further analyzed with subtype-specific primers. From December 2011 to May 2012, 963 adult pigs were sampled, with a prevalence of IgG antibody of 60% (573/958) and 4% (42/963) of animals positive by PCR. Subtype identification from the 42 PCR positive animals were 25 (60%) pandemic H1N1, 8 (19%) H3, 6 (14%) seasonal H1, and 4 (10%) un-subtypable, on which sequencing is presently underway. H1 pandemic/H3 coinfection was noted in 3 (7%) samples. Pigs in the study area are frequently infected with influenza A viruses, primarily human subtypes, providing ample opportunity for coinfections and reassortants.

## 446

### SEVERITY OF ILLNESS AND CHRONIC MALNUTRITION IN CHILDREN WITH ROTAVIRUS-ASSOCIATED DIARRHEA IN GUATEMALA

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Rotavirus is the most important cause of severe diarrhea in young children in developing countries. Malnutrition impairs immune responses and may contribute to severity of diarrheal illness. We investigated the relationship between stunting, an indicator of chronic malnutrition, and severity of rotavirus-associated diarrhea in children <5 years in Guatemala. We enrolled children born after June 1st 2009, who were hospitalized

or treated in the emergency department for diarrhea in 4 hospitals in Guatemala, December 2012-April 2013. Stool samples were assayed for rotavirus using ELISA. Heights and weights were measured at presentation; growth stunting was defined as height for age Z-score (HAZ) <-2 (per WHO standards). Based on the 20-point Vesikari severity score, patients were categorized as having severe (≥11) vs. mild/moderate (<11) illness. Logistic regression models were constructed for calculating odds ratios (OR) for relationships between stunting and severity of illness among cases positive for rotavirus, adjusting for site (Guatemalan department), gender, age, history of breastfeeding, rotavirus vaccination, socioeconomic and sanitation variables. 231 children tested rotavirus positive. Among them, median age was 12 months (IQR 9-17), 72 (31%) met criteria for stunting, and 108 (47%) received at least one dose of the rotavirus vaccine, 209 (90%) had a history of being breastfed. 179 diarrhea cases (77%) were categorized as severe, of which 60 (34%) were stunted. We found a higher odds of severe diarrhea among children with stunting (aOR=2.6, 95%CI 0.9-7.7, p=0.07). Chronic malnutrition was associated with risk of developing severe rotavirus disease.

## 447

### RIFT VALLEY FEVER SEROPREVALENCE IN COASTAL KENYA

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Rift Valley fever virus (RVFV) causes severe disease in both animals and humans that can result in significant economic and public health harm. The objective of this study was to measure the prevalence of RVFV exposure during 2009-2011 in six coastal Kenyan villages, and link seropositivity to demographics, socioeconomic status, mosquito density, and other risk factors. Demographic, household inventory and exposure questionnaires were administered to all participants. Sera were tested for anti-RVFV IgG via standard ELISA. Bivariate relationships for each potential predictor of RVFV seropositivity were assessed using a $\chi 2$ test. Multivariable logistic regression was used to further test predictor variables for association with seropositivity. Overall, 2,871 sera were tested; 51 (1.8%; 95%CI 1.3-2.3) were RVFV seropostive. Rates differed significantly among villages, with Jego having the highest rate (18/300; 6.0%; 95%CI 3.6-9.3) and Magadzoni having the lowest (0/248). Other village rates were as follows: Vuga 1.0% (8/835; 95%CI 0.4-1.9); Kinango 1.0% (5/524; 95%CI 0.3-2.2); Nganja 1.7% (7/404; 95%CI 0.1-3.5); and Milalani 2.3% (13/560; 95%CI 1.2-3.9). Adults were more likely to be seropositive than children (p<0.001), but there were no statistically significant differences between genders. Those who owned land were also more likely to be seropositive (p<0.001). There was not a significant correlation between livestock or *Culex* density and human seroprevalence. However, the two highest prevalence villages experienced periodic flooding during this time, and the highest prevalence village, Jego, was adjacent to a herding community that had exceptionally high livestock and cattle numbers. Overall, Rift Valley Fever exposure is low in coastal Kenya, although significant village level variation is present. As in other regions, adults are more likely to be exposed.

## 448

### MOLECULAR CHARACTERIZATION OF GROUP C ORTHOBUNYAVIRUSES ISOLATED IN PERU

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Group C viruses are a complex of viruses in the genus *Orthobunyavirus*, family *Bunyaviridae*. These viruses are associated with human febrile disease in tropical and subtropical areas of South and Central America. While numerous group C orthobunyaviruses have been isolated from mosquitoes, animals, and humans, genetic analysis of these viruses is limited. Since the 1990s, we have conducted passive surveillance for febrile disease in Peru. During this time, 65 virus isolates from febrile patients in the northern and southern Peruvian Amazon were identified as group C orthobunyaviruses using an immunofluorecent assay. To further characterize these isolates, a 500bp region of the S segment was sequenced. Pairwise sequence analysis of the clinical isolates showed nucleotide identities ranging from 71% to 100% and deduced amino acid sequence identities ranging from 74% to 100%. For comparison, we sequenced prototype strains from the following group C antigenic groups: Caraparu virus (CARV), Murutucu virus (MURV), Oriboca (ORIV), Marituba (MTBV) and Apeu (APEUV). Sequence comparison of the clinical isolates with the prototype strains showed that 48 isolates had 85 - 88% nucleotide and 96 - 98% amino acid identity with CARV and 17 isolates had 82 - 86% nucleotide and 96 - 97% amino acid identity with MURV, ORIV, APEUV and MTBV. Based on phylogenetic analysis, sequences could be separated into two clades. One clade contained viruses from both the northern and southern Peruvian Amazon and the prototypical CARV strain, the other clade contained clinical isolates from the northern Peruvian Amazon and the prototypical ORIV, MURV, APEU, and MTBV strains. These results demonstrate the genetic relationships of group C viruses circulating in the Peruvian Amazon and the genetic divergence of group C viruses within the northern Amazon.

## 449

### ANALYSIS OF BLOOD STAGE MALARIA IMMUNITY INDUCED BY CHEMICALLY ATTENUATED PARASITES

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The development of vaccines for malaria has focused on the induction of antibodies to parasite surface antigens, most of which are highly polymorphic. An alternate strategy has evolved from observations that low-density infections curtailed by drug treatment can induce antibody-independent immunity to different strains. To test this strategy as a vaccine approach we treated parasitized red blood cells from the rodent parasite, *Plasmodium chabaudi*, 98 % of which were at the early 'ring' stage, with seco-cyclopropylpyrroloindole analogs, which covalently bind parasite DNA, and administered these to mice without adjuvant. DNA from the vaccine could be detected in the blood for over 110 days although we have not been able to visualize intact parasites. A single vaccination induced profound immunity to different malaria parasite species. Immunity was mediated by CD4+ T cells and was dependent on the red cell membrane remaining intact. T cells from vaccinated mice responded *in vitro* to both homologous and heterologous strains. Activation of T cells was antigen-specific since adoptively transferred ovalbumin-specific (OTII) cells were not activated by vaccination. Vaccine-induced T cells expressed

activation markers within 5 days of vaccination and contained intracellular gamma-interferon. Immunity could not be transferred with antibodies from vaccine-protected mice. Immunity persisted for at least 6 months. The human parasite, *P. falciparum*, could also be attenuated and we are now undertaking a human Phase I trial using these parasites prepared in our GMP-compliant facility. We believe that this approach will have relevance to the development of other parasite vaccines.

## 450

### HUMORAL RESPONSES AGAINST *PLASMODIUM FALCIPARUM* AFTER CHLOROQUINE PROPHYLAXIS AND SPOROZOITE (CPS) IMMUNIZATION AND THEIR CORRELATION WITH PROTECTION IN HUMAN VOLUNTEERS AND A NOVEL MURINE CPS-MODEL

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Malaria vaccines are urgently needed for better control and eventually elimination. One of the major hurdles in vaccine design and development is incomplete understanding of protective immunity. Protection against malaria challenge infection can be induced by repeatedly exposing healthy human volunteers to *Plasmodium falciparum* infected mosquito bites during a prophylactic course of chloroquine (Chemoprophylaxis and Spozoroites, CPS). This regime allows full exposure to the clinically silent liver-stage of infection during the immunization period, whilst limiting exposure to the pathogenic blood-stage parasite. Antimalarial antibody responses have long been known to contribute to protective immunity against the disease, so we wanted to understand the development of humoral responses during repeated malaria infection under drug cover. We investigated the kinetics and antigen-specificity of antibodies and memory B-cells (MBC) during immunization and their correlation with protection against challenge infection by performing standardized MBC ELISpot and ELISA on sequential peripheral blood mononuclear cell and plasma samples from 38 healthy Dutch adult volunteers enrolled in two clinical CPS-immunization trials. Antigen specificity was assessed using nine antigens representing the different life-cycle stages of the malaria parasite. We demonstrate, for the first time, that CPS-immunization induces MBCs and antibodies specific for liver- and cross-stage antigens, which are gradually acquired over the course of CPS immunization and boosted upon malaria challenge. Importantly, CPS-induced MBC responses are more stable and more efficiently boosted after challenge, as compared with plasma antibody titres. Levels of MBC and antibodies to CSP, LSA-1, AMA-1 and MSP-1, the most dominant humoral responses during malaria infection, correlate with the degree of parasite exposure during immunization, but are not predictive of protection. Therefore, to determine the role of B cells in protection to malaria challenge following CPS immunization, we developed a novel experimental mouse model that replicates our human clinical trials. We show that B cells are absolutely essential for protection to malaria challenge following CPS-immunization in mice. In the future this model will allow us to dissect protective antigens to support the rational development of novel malaria subunit vaccines.

## 451

### NATURALLY ACQUIRED *PLASMODIUM FALCIPARUM* RH5-SPECIFIC IGG ANTIBODIES CORRELATE WITH PROTECTION FROM MALARIA

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Vaccine strategies targeting *Plasmodium falciparum* asexual blood stages, which cause the clinical manifestations of malaria, have yet to show protective efficacy in clinical trials. *P. falciparum* reticulocyte-binding protein homologue 5 (PfRH5), an essential merozoite protein involved in erythrocyte invasion, is an attractive candidate for a blood-stage vaccine. We investigated the association between naturally acquired PfRH5-specific IgG present before the malaria season in Mali and the prospective risk of malaria using time from first PCR-confirmed *P. falciparum* blood-stage infection to first malaria episode as the primary outcome. *P. falciparum* infections were detected by retrospective PCR analysis of dried blood spots which had been collected every 2 weeks for 7 months, and clinical malaria episodes were detected by weekly active surveillance and self-referral. Baseline IgG responses to PfRH5 and another blood-stage antigen *P. falciparum* apical membrane antigen 1 (PfAMA1) were determined by ELISA in 342 individuals aged 6 months to 25 years who began the study free of blood-stage *Plasmodium* infection. 284 individuals subsequently became infected with *P. falciparum* by PCR detection during the ensuing malaria season. A significant delay in median time from blood-stage infection to first malaria episode was observed among individuals with positive IgG responses to PfRH5 (n=48; 71 days; 95% CI, 21-229 days) compared to individuals with negative responses (n=236; 18 days, 95% CI, 14-26 days) by log-rank test ($P$=0.001). After adjustment for age, sickle cell trait, anemia, and PfAMA1 IgG levels using a Cox proportional hazards model, the protective effect of PfRH5-specific IgG on malaria risk remained (HR 0.62, 95% CI, 0.42–0.94, $P$=0.02). PfRH5 IgG levels did not associate with reduced parasite density or multiplicity of infection at the first malaria episode. We also assessed the ability of naturally acquired PfRH5-specific antibodies to neutralize parasite invasion of erythrocytes *in vitro* using growth inhibition assays. These findings provide the first evidence for the role of naturally acquired PfRH5-specific antibodies in clinical immunity to *P. falciparum* malaria. Our methodology for assessing malaria risk improves the ability to detect associations between immune responses to blood-stage infection and clinical protection and may prove useful for evaluating potential correlates of blood-stage immunity.

## 452

### ANTIBODIES TO SCHIZONT EGRESS ANTIGEN-1 (PFSEA-1) BLOCK SCHIZONT EGRESS FROM *FALCIPARUM* INFECTED RBCS

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We discovered PfSEA-1 using a differential screening approach contrasting plasma from children who were resistant or susceptible to *falciparum* malaria. Antibodies to the immunorelevant region of PfSEA-1 (rPfSEA-1A) predict resistance to severe disease in two yr olds and vaccination with rPbSEA-1A protects mice from *Plasmodium berghei* ANKA challenge. To further characterize PfSEA-1, we performed growth inhibition assays (GIA) and immunolocalization studies. For GIA assays, parasites were synchronized, plated at 0.3-0.4% *parasitemia*, and cultured to obtain mature trophozoites. Mature trophozoites were cultured in the presence of anti-rPfSEA-1A or pre-immune mouse sera. Parasites were cultured for 24 hrs and ring stage parasites were enumerated. Anti-PfSEA-1A inhibited parasite growth by 58-74% across three parasite strains (3D7, W2 and D10) compared to controls (all $P < 0.009$). In confocal and immunogold EM studies, PfSEA-1 localized to the schizont/parasitophorous vacuole membrane, Maurer's clefts and the inner leaflet of the RBC membrane. This localization of PfSEA-1 was not consistent with a role in RBC invasion; rather it suggested a role in parasite egress from iRBCs. To determine the mechanism of growth inhibition we performed schizont arrest assays (SAA) using anti-rPfSEA-1A. For SAA, parasites were synchronized three times using sorbitol, plated at 3.5% *parasitemia*, and cultured to obtain early schizonts. Early schizonts were cultured in the presence of anti-rPfSEA-1A or pre-immune mouse sera. Schizonts were enumerated at 12 hrs post-treatment and the percent of schizonts arrested calculated. Anti-rPfSEA-1A dramatically inhibited schizont egress resulting in 4.3-6.8 fold higher proportion of schizonts across three parasite strains compared to controls (all $P < 0.009$). Our data support PfSEA-1 as a novel vaccine candidate for pediatric *falciparum* malaria. By blocking schizont egress, PfSEA-1 may synergize with vaccines targeting hepatocyte and red cell invasion.

## 453

### HETEROLOGOUS PROTECTION AGAINST MALARIA IN A SUBSET OF VOLUNTEERS AFTER IMMUNIZATIONS WITH *PLASMODIUM FALCIPARUM* SPOROZOITES UNDER CHLOROQUINE PROPHYLAXIS

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We previously reported that long-lasting homologous protection against *falciparum* malaria can be induced in malaria naive subjects on chloroquine prophylaxis, by bites of *Plasmodium falciparum* infected mosquitoes using the ChemoProphylaxis and Sporozoites (CPS) protocol. In a clinical follow-up study we assessed whether CPS-immunized and protected subjects were protected against a heterologous strain. Twelve CPS-immunized subjects exposed to either 3x15, 3x10 or 3x5 NF54 - infected mosquitoes and protected against a homologous NF54 (West-Africa) together with five malaria-naïve controls were challenged by the bites of 5 mosquitoes infected with the heterologous *P. falciparum* clone NF135. C10 originating from Cambodia at 14 months after the last immunization. The primary outcome was time to parasitaemia after challenge infection assessed by microscopy and qPCR. Two out of 12 previously CPS-immunized and protected subjects against NF54 were fully protected against a heterologous NF135.C10 re-challenge of which one subject was previously immunized with 3x 15 mosquito bites and one subject with 3x10 mosquito bites. The remaining volunteers developed *parasitemia* at a significant later time point (median pre-patent period by qPCR 10.5 days) than the controls who all had a pre-patent period (qPCR) of 7 days. In conclusion, CPS immunization can induce heterologous protection against a geographically and genetically distinct *P. falciparum* NF135 clone at 14 months after the last immunization with NF54. The pre-patent period in partially protected subjects was significantly delayed. Unprotected subjects showed strong and short adverse reactions to heterologous challenge which may be immune-mediated in response to blood stages and is an indication to partial protection. These initial data provide a basis to further explore CPS-induced heterologous protection, critical for clinical development of whole sporozoite-based vaccines.

## 454

### ANTIBODY PROFILES INDUCED BY CONTROLLED HUMAN MALARIA INFECTIONS IN HEALTHY VOLUNTEERS UNDER CHLOROQUINE PROPHYLAXIS

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Complete sterile protection to *Plasmodium falciparum* (Pf) infection can be experimentally induced by controlled human malaria infections in healthy volunteers under chloroquine prophylaxis, through immunization with sporozoites delivered by bites of infected mosquitoes (CPS protocol). To characterize the profile of induced antibody specificities in blood samples collected at preimmunization and pre-challenge, we used a proteome microarray containing 811 Pf antigens recognized by plasma from naturally exposed individuals from Africa and by volunteers immunized with irradiated sporozoites. CPS-immunized and protected individuals generated antibodies against 174 Pf antigens, whereas reactivity of mock-immunized controls was negligible. Predominant reactivity was found against the pre-erythrocytic antigens circumsporozoite protein (CSP) and liver stage antigen 1 (LSA1), and to a lesser extent against MSP2, MSP8, MSP10, ApiAP2, and the hypothetical PF07_0053 antigen. In comparison, specimens of semi-immune adults from western Kenya showed a different profile with emphasis on blood stage antigens and scarce reactivity against LSA1 and CSP. The uniformly elevated reactivity of LSA1 and CSP in all of the protected individuals in this study highlights the potential importance of these two pre-erythocytic antigens in the protective response induced by CPS immunization. These data provide insight and guidance to pre-erythrocytic vaccine development by elucidating antigen targets involved in protective immunity against sporozoite and liver stages, however, the array used for this study is enriched in blood stage antigens and the liver stage proteome is underrepresented. Due to the importance of liver stage immunity associated with CPS-induced protection we are in the process of completing the Pf 3D7 proteome microarray to get a more complete understanding of pre-erythocytic immunity induced by the CPS protocol. This next generation Pf proteome array will contain more than 9,000 features covering all 5,400 encoded proteins, included hundreds of pre-erythocytic antigens that have never before been probed.

## 455

### EFFECTIVENESS OF INSECTICIDE-TREATED BEDNETS TO REDUCE THE RISK OF MALARIA IN CHILDREN IN AN AREA OF MALAWI WITH SIGNIFICANT PYRETHROID RESISTANCE

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Insecticide-treated bednets (ITNs) are the cornerstone of malaria control in sub-Saharan Africa, but ITN effectiveness may be compromised in areas of pyrethroid resistance. Between 2011 and 2013 in Machinga District, Malawi, WHO resistance assays with *Anopheles funestus*, the predominant malaria vector, found mortality at 24 hours to be 0-48% for deltamethrin and 72% for permethrin. We conducted a cross-sectional survey in March and April 2012, prior to the start of an observational cohort study, to calculate the protective effectiveness (PE) of ITNs among children who did and did not sleep under ITNs. All households in six rural villages of Machinga District were censused and children aged 6 to 59 months were invited to participate. At enrollment, ownership, use, age and condition of ITNs was assessed by caregiver verbal report, and children provided finger-prick blood samples for polymerase chain reaction (PCR) testing of malaria *parasitemia*. Out of 1,667 participants, 1,200 (72%) met the inclusion criteria and provided written consent for enrollment. A total of 443 (37%, 95% confidence interval [CI] 34-40%) children were parasitemic by PCR. ITNs were used by 516 (45%) children, untreated bednets (UTNs, defined as any ITN >36 months old) were used by 388 (34%) and 253 (22%) children reported not using any bednet the night before the survey. Holes were noted in the ITNs of 302 (59%) children and in the UTNs of 304 (78%) children (p<0.001). Using a log-binomial model controlling for child age, household wealth, maternal education and the number of bednets within a 300m radius of the child's household, and accounting for clustering by household, the PE of ITNs in reducing the risk of malaria compared to no bednets was 25% (95% CI 10-37%) and the PE of UTNs compared to no bednets was 30% (95% CI 14-42%). Despite moderate to high pyrethroid resistance in this area, ITNs were effective at reducing the risk of malaria *parasitemia* in children 6 to 59 months old compared to no bednets. UTNs, however, were equally effective at reducing the risk of *parasitemia* in this age group, raising questions as to the mechanism by which ITNs may be protecting children against malaria.

## 456

### THE PREVALENCE, CLINICAL FEATURES AND OUTCOMES OF PUTATIVE HEMOGLOBINURIA AMONG EAST AFRICAN CHILDREN RECRUITED TO THE FEAST TRIAL WITH SEVERE FEBRILE ILLNESSES

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Blackwater fever (BWF) remains poorly described among African children. Even less is known in terms of geographical distribution, innate characteristics, immunological, and infectious causation of this syndrome in children. We describe Dark Urine Syndrome (DUS) in East African children to understand its distribution geographically, with and without malaria, and associated complications of severe anaemia, jaundice, renal impairment and their outcomes in the six FEAST trial sites in East African countries involved. The analysis of FEAST data was done using STATA version 11.0. Continuous variables among the DUS were compared with those in the controls using the student's t-test and by ANOVA, while categorical variables were analysed using X2 - test. P-values of <0.05 were considered statistically significant and included in the multivariate analysis for risk factors for dark urine. The median age of children with DUS was 24 months (IQR 13, 38) without male predominance. A majority of patients with DUS 318/394 (81.0) were from eastern Uganda, where malaria transmission is very high; 256/318 (80.5%) also had clinical jaundice. Quality-controlled slide and/or rapid diagnostic tests for *P. falciparum* infection was positive in only 147/300 (49.0%) of DUS patients. Severe anaemia (Hb <5g/dL) complicated 238/310 (77.0%) of DUS compared to 480/1480 (32.4%) in the non-DUS (ND) group P 5mmol/L) was predominant in DUS 204/309 (66.0%) vs. 560/1450 (38.6%) in ND group P 20mmol/L) was marked in DUS group 123/187 (65.8%) vs 140/847 (38.6%) in ND group P <0.001. Mortality was similar in the DUS (12.3%) and ND group (9.9%) P = 0211. DUS in East African children is a complex phenomenon without an apparent single aetiology or pathophysiology. The syndrome was not limited to malaria alone as previously thought and without a male predominance G6PD as a major cause was highly unlikely. These novel findings of a high risk of renal injury and DUS indicate substantial geographical differences in the spectrum of severe disease across East Africa.

## 457

### PREGNANCY MALARIA AND INFANT SUSCEPTIBILITY TO CLINICAL MALARIA IN AN AREA OF SEASONAL, INTENSE MALARIA TRANSMISSION

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Malaria in pregnancy is associated with poor pregnancy outcomes. In a previous study in Tanzania where transmission is perennial and intense, we observed that placental malaria modifies the susceptibility of infants to malaria infection, depending on the mother's parity. To assess this relationship in Mali where transmission is highly seasonal, pregnant women in Ouelesssebougou were enrolled and followed up to delivery, and their singleton children born between January 2011 and November 2012 were followed up to December 31st, 2012. Cox proportional hazards model was used to compare risk of clinical malaria episodes in the offspring, adjusted for potential confounders such as parity, use of insecticide impregnated nets (ITN), hemoglobin type, location and season. A total of 425 mother-offspring pairs were analyzed. Compared to other infants, infants born from mothers who had pregnancy malaria were 58% more likely to experience clinical malaria at a younger age (adjusted hazard ratio [AHR] = 1.58 (95% CI, 1.17 - 2.13). Although malaria in pregnancy is more frequent during first pregnancy, the risk of clinical malaria in firstborn children was lower compared to other children (AHR = 0.65 (95% CI 0.45 - 0.93). The risk of the clinical malaria was also lower with regular use of ITN (AHR = 0.73 (95% CI 0.54 - 1.00) and in infants with hemoglobin S (AHR = 0.54, (95% CI 0.29- 0.98). In conclusion, malaria in pregnancy is associated with increased risk of the clinical malaria in the offspring. First pregnancy, hemoglobin S and regular use of ITN reduce the risk of clinical malaria in offpring.

## 458

### ESTIMATES OF THE RISK OF PLACENTAL INFECTION AND BURDEN OF LOW BIRTHWEIGHT ATTRIBUTABLE TO *PLASMODIUM FALCIPARUM* MALARIA IN AFRICA IN 2010

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*Plasmodium falciparum* infection during pregnancy leads to adverse outcomes including low birthweight (LBW). Women acquire immunity to malaria in pregnancy over consecutive pregnancies and are most susceptible during their first pregnancy. Using a model of the parity-dependent immunity acquisition and estimates of the geographical distribution of fertility and P.*falciparum* transmission, we estimate the number of women who could be expected to experience placental infection in the absence of pregnancy-specific interventions in Africa. By fitting our model to patterns of excess LBW risk in women experiencing placental infection in Kilifi, Kenya and Ifakara, Tanzania, we then estimate the burden of malaria-attributable LBW. Without pregnancy-specific protection we estimate that, in Africa in 2010, 11.4 (95% CrI 10.7-12.1) million pregnancies would have experienced placental infection at some stage of gestation, accounting for 41% of the total 27.6 million live-births. Combining this with our estimated relationship between placental infection and LBW, we found the potential LBW burden due to placental malaria was 900,000 (95% CrI 530,000-1,240,000) LBW deliveries per-year. The time at which the placenta becomes susceptible to infection, around the end of the first trimester, is a key period when we estimate 65% (95% CrI 61%-70%) of the potentially infected pregnancies first experience infection. Primigravidae experience a disproportionate 39% (95% CrI 33%-46%) of the total potential placental malaria-attributable LBW burden. These are the only contemporary estimates of the distribution of risk and associated LBW burden of malaria in pregnancy in Africa. They suggest that the risk of placental infection across Africa in unprotected women remains large. Prevention of malaria pre-conception or very early in pregnancy is predicted to have a major impact upon reducing LBW, particularly in primigravidae. Lifetime risk of LBW changes gradually with transmission, highlighting the need to maintain protection as transmission falls and the incremental benefit of malaria elimination.

## 459

### EFFECTS OF RESTRICTING ARTEMISININ-BASED COMBINATION THERAPY TO TEST POSITIVE MALARIA IN A HIGH-TRANSMISSION SETTING IN GHANA: A CLUSTER-RANDOMIZED TRIAL

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The policy of test and treat malaria is replacing presumptive treatment in many endemic countries. There is however very limited data on the effect of test and treat policy on the incidence of malaria, anaemia and severe febrile illness in high-transmission settings. We conducted a cluster randomized trial to compare the effect of test and treat versus presumptive treatment in a high transmission setting in Ghana. Thirty two health centres were randomly allocated to test treat (TT) or presumptive treatment (PT) arms. Children aged 2-24months living around health centre were enrolled and the incidence of malaria, anaemia and severe febrile illness were measured by passive and active surveillance over 24 months. A total of 3046 children were enrolled in 32 health centres. The incidence of malaria in the TT arm was 696/1000 pyrs (95% CI 557, 870) and 800/1000 pyrs (700, 914) in the PT arm (P=0.56). The incidence of first or only episode of malaria after the first episode of febrile illness was 558/1000 pyrs (95% CI 434, 718) in the TT arm and 673/1000 pyrs (544, 833) in the PT arm (p=0.34); the incidence of anaemia was 82/1000 (63, 107) versus 92/1000 (71, 119) (P=0.61); incidence of severe febrile illness was 129/1000 (82, 210) versus 151/1000 (95, 250)(P=0.84). Children in the PT arm were more likely to be prescribed ACT than children in the TT arm (81.6% versus 72.1%; P=0.01). Restricting the use of artemisinin combination therapy to test positive malaria is appropriate even in high transmission settings.

## 460

### SURVEILLANCE OF VECTOR POPULATIONS AND MALARIA TRANSMISSION DURING THE 2009/10 EL NINO EVENT IN THE WESTERN KENYA HIGHLANDS: OPPORTUNITIES FOR EARLY DETECTION OF MALARIA HYPER-TRANSMISSION

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Vector control in western Kenya highlands has resulted in a significant reduction of malaria transmission and a change in the vectorial system. Surveillance and monitoring is an important component of early transmission risk identification and management. However, below certain disease transmission thresholds, traditional tools for surveillance such as entomological inoculation rates may become insensitive. This study was undertaken toexamine the possibility of using a rapid diagnostic kit for the detection of anti-malaria immune markers circum-sporozoite protein antibodies and merozoite surface protein antibodies as an early indicator of transmission change. Indoor resting female adult malaria vectors were collected in four selected villages in U-shaped (Iguhu and Emutete) and the V-shaped valleys (Marani and Fort Ternan) for eight months. *Anopheles gambiae* complex was identified by PCR. Blood samples were collected from children 6-15 years old and exposure to malaria was tested using a circum-sporozoite protein and merozoite surface protein immunchromatographic rapid diagnostic test kit. Sporozoite ELISA was conducted to detect circum-sporozoite protein, later used for estimation of entomological inoculation rates. An upsurge in antibody levels was first observed in October 2009 in all the study sites while *Plasmodium falciparum* sporozoites were first observed in December 2009 at Iguhu and February 2010 at Emutete. Despite the upsurge in Marani and Fort Ternan no sporozoites were detected throughout the study period. The antibody-based assay was more sensitive and had much earlier transmission detection ability than the sporozoite-based assay. The proportion of An. *arabiensis* among *An. gambiae s.l.* ranged from 2.9-66.7% indicating a rearrangement of the sibling species of the *An. gambiae* s.l complex. In conclusion, the rapid diagnostic kit with molecular markers should be used with other malaria surveillance tools such as the climate based model in order for it to be efficient in the U shaped valleys which were the hotspots for malaria transmission.

## 461

### MAJOR BURDEN OF SEVERE ANEMIA FROM NON-*FALCIPARUM* MALARIA SPECIES

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The burden of anemia attributable to the non-*falciparum* malarias in regions with *Plasmodium* co-endemicity is poorly documented. We

compared the hematological impact of all endemic *Plasmodium* species in southern Papua, Indonesia. Prospectively collected clinical and laboratory data were linked for all patients presenting to the main referral hospital over a 5 year period. Of 497,983 patients presenting to hospital (446,543 outpatients and 51,440 inpatients), a total of 115,972 (23.3%) were associated with a hemoglobin measurement. Of these 37,249 (32.1%) were infected with *Plasmodium* (*P. falciparum* n=23,716 [63.7%], *P. vivax* n=9,791 [26.3%], mixed infection n=2,931 [7.9%] and *P. malariae* n=790 [2.1%]). Patients with *P. malariae* had the greatest mean reduction in hemoglobin (-1.52 g/dL, 95% confidence interval [CI] -1.70, -1.35 g/dL) followed by those with mixed (-1.47 g/dL, 95%CI -1.58, -1.37 g/dL), *P. falciparum* (-0.91 g/dL, 95%CI -0.95, -0.87 g/dL) and *P. vivax* infections (-0.87 g/dL, 95%CI -0.93, -0.81 g/dL); p for all comparisons <0.001). Severe anaemia (Hb<5 g/dL) was present in 5896 (5.1%) patients, and was associated with a significant increased risk of death (OR = 4.77 [95%CI 4.23-5.36], p<0.001). Patients with mixed infection were at greatest risk of SMA (Adjusted Odds Ratio [AOR] 4.11, 95%CI 3.64,4.64; AORs for *P. falciparum*, *P. vivax* and *P. malariae* were 2.43 (95%CI 2.27,2.61) 2.43 (95%CI 2.23,2.66) and 2.74 (95%CI 2.12,3.53) respectively, p for all comparisons <0.001). *P. vivax* infection was the most common cause of malaria in the first year of life and accounted for 23.4% (95%CI 19.6,27.1%) of severe anemia in this age group. Overall, 9.7% (95%CI 8.8,10.6%) of severe anemia was attributable to non-*falciparum* infections compared with 11.7% (95%CI 10.6,12.7%) for *P. falciparum* monoinfections. In Papua, a resource poor setting, *P. vivax* is the dominant cause of SMA in infancy and mixed *P. vivax*/*P. falciparum* infection is associated with substantially greater hematological impairment than either species alone. These findings highlight the public health importance of integrated genus-wide malaria control strategies in areas of *Plasmodium* co-endemicity.

## 462

### FEBRILE ILLNESS AND PRO-INFLAMMATORY CYTOKINES IN THE FIRST YEAR OF LIFE PREDICT IMPAIRED CHILD DEVELOPMENT IN BANGLADESHI INFANTS LIVING IN POVERTY

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An estimated one-third of children in low-income and middle-income countries fail to meet their full developmental potentials. The first year of life is a period of critical and rapid brain development and is also when most of the morbidity and mortality from infection is suffered. Recurrent infection results in stunted growth and stunting is a known predictor of cognitive impairment. It is, however, unknown whether infection has an independent role in cognitive development through mechanisms such as chronic inflammation. Studies in preterm infants have linked elevated levels of inflammation-related proteins near the time of birth to cognitive dysfunctions years later. We, therefore, hypothesized that clinical and biomarkers of inflammation in the first year of life can predict cognitive, language, and motor impairments in children living in an urban slum in Bangladesh. A cohort of 398 children in Dhaka, Bangladesh was observed from birth until 30 months of age. Febrile illness was used as a clinical marker of inflammation and elevated concentrations of inflammation-related cytokines (IL-1β, IL-6, TNF-α, IL-4, IL-10) in 6 month sera were used as biomarkers of inflammation. Psychologists assessed cognitive, language, and motor development using the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III) at 12 months, 24 months, and 30 months of age. Associations between febrile illness and elevated cytokine levels with developmental outcomes were evaluated using linear mixed models. Cognitive, language, and motor scores all declined significantly over time (all $p < 0.0001$). Duration of febrile illness during the first year of life was associated with impairments in language and motor development ($p = 0.003$ and 0.0003). Elevated levels of the proinflammatory cytokines IL-1β and IL-6 were significantly associated with impairments in motor development, while elevated levels of the immunomodulatory cytokine IL-4 were associated with higher cognitive scores (all $p < 0.05$). Our work has identified immune correlates of developmental outcomes in children, which could serve as both prognostic markers as well as potential immunological targets for interventions to prevent developmental impairment in at-risk children.

## 463

### ALCOHOL, SOCIAL INSTABILITY, AND DEFAULT FROM MULTIDRUG RESISTANT TUBERCULOSIS TREATMENT IN RURAL SOUTH AFRICA

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Default from multidrug-resistant tuberculosis (MDR TB) treatment remains a major barrier to cure. As TB treatment programs transition to managing MDR TB entirely in the outpatient setting, the patients at highest risk for default during out-of-hospital treatment must be identified so that efforts to increase adherence can focus on them. In this study, we retrospectively analyzed a cohort of 225 patients who initiated MDR TB treatment at a rural TB hospital in the Western Cape Province, South Africa, from 2007 through 2010. We performed multivariable proportional hazards regression analysis to identify baseline risk factors for default, and we compared default rates, stratified by key risk factors, across relevant time intervals, seeking to understand high-risk time periods for default in relation to the hospitalization period. Fifty percent of the patients in this cohort were cured or completed treatment, 27% defaulted, 14% died, 5% failed treatment, and 4% transferred out. 63% of patients reported recent alcohol use. Younger age (hazard ratio [HR]=1.03 [CI 1.01-1.06]/year), informal housing (HR=2.8 [1.5-5.0]), lack of steady employment (HR=2.6 [1.1-5.9]), Cape-Coloured ethnicity (HR=2.3 [1.1-5.0]), and alcohol use (HR=2.1 [1.1-4.0]) were associated with default ($P<0.05$). Defaults occurred throughout the first 18 months of the two-year treatment course but were especially frequent among alcohol users after discharge from the initial four-to-five-month in-hospital phase of treatment, with the highest default rates occurring among alcohol users in the first two months after hospital discharge. We conclude that default is a major barrier to cure of MDR TB in this rural farming population and that interventions designed to increase adherence among young, economically-unstable patients and alcohol users could potentially increase cure rates. In particular, alcohol abusers need extra support during outpatient treatment in order to remain adherent.

## 464

### UTILITY AND CONCORDANCE OF TST, IGRA, AND IP-10 IN DETECTING NEW *MYCOBACTERIUM TUBERCULOSIS* INFECTION IN HOUSEHOLD CONTACTS FROM VITÓRIA, BRAZIL

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Tuberculosis (TB) continues to be a major cause of morbidity and mortality worldwide, most significantly in resource-limited settings. Although most individuals enter a state of clinical latency upon infection with *Mycobacterium tuberculosis* (MTB), about 5% develop progressive TB within two years of infection. To meaningfully interrupt disease transmission, we need more accurate and efficient identification of individuals at greatest risk of progression, i.e. those newly infected. To evaluate the use of tuberculin skin testing (TST) and the interferon-γ (IFNγ) release assay (IGRA) in those recently exposed to MTB, we enrolled 146 household contacts (HHC) of TB cases in Vitória, Brazil. TST was performed post-exposure and if negative, again at 8-12 weeks. HHC were classified as initially TST-positive (TSTpos), persistently TST-negative (TSTneg), or TST-converters (TSTc), the latter representative of new infection. Additional testing at 8-12 weeks included IGRA and measurement of IFNγ inducible protein-10 (IP-10), a molecule downstream of IFNγ signaling hypothesized to improve sensitivity of IGRA testing. There was generally poor agreement between TST and IGRA results (kappa=0.35). Of the 24/70 individuals who converted their TST from negative to positive, only 33.3% tested IGRA-positive. This is compared to 65.3% of TSTpos HHC who tested IGRA-positive, suggesting there may be a delay in IGRA conversion beyond 8-12 weeks post-exposure. IGRA testing differentiated TSTpos from TSTneg ($p<0.001$) but there was no difference between TSTneg and TSTc ($p=0.25$). Results were similar for IP-10. Lowering the cutpoint for a positive IGRA from 0.35 to 0.15 IU/ml increased the sensitivity of detecting TSTc from 33.3% to 45.8% although decreased specificity from 84.8% to 78.3%. Similarly, measuring IP-10 improved sensitivity for detecting TSTc and TSTpos, however, compromised specificity. Our results suggest that using IGRA and/or IP-10 for diagnosing new MTB infection may be limited by a delayed conversion to test positivity.

## 465

### ASSOCIATIONS BETWEEN INFLUENZA ACTIVITY IN KENYA AND TEMPERATURE, RAINFALL, SPECIFIC HUMIDITY AND SOLAR RADIATION, 2010-2011

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Temperature and humidity are thought to be associated to seasonal influenza circulation in the temperate regions, but this relationship is less clear in the tropics. We analyzed the association between meteorological parameters and influenza frequency in 10 sentinel surveillance sites in Kenya (8 public hospitals and 2 refugee camps) that include tropical, arid and temperate-like climate zones. We obtained the weekly proportion of samples testing positive for influenza from persons visiting outpatient clinics for Influenza-like Illness (ILI) and those hospitalized with Severe Acute Respiratory Illness (SARI) during 2010 to 2011. Night Land Surface Temperature (a proxy for minimum air temperature), rainfall, specific humidity (SH) and solar radiation were retrieved from NASA's satellite and land model. For each site, we modeled the proportion of samples testing positive using binomial regression with the meteorological parameters entered via backward selection. We found SH was significantly associated ($p<.05$) either positively or negatively in all sites (negative in 8 sites, positive in 2 sites). The 2 sites with positive association were Mombasa (coastal region) and Dadaab (refugee camp), which had higher average SH (16 and 14 g/kg, respectively) compare to other sites (average 12 g/kg). In addition, influenza positivity was also significantly associated with: increasing rainfall and solar radiation in Mombasa; decreasing solar radiation in Kakuma Camp; decreasing temperature in Kakamega district. When the regression was applied to all areas at once, influenza positivity was significantly associated ($p<.05$) only with decreasing specific humidity. In conclusion, our findings underscore the link between influenza positivity and SH across Kenya's diverse climatic zones. The positive relationship with SH in the coastal Mombasa is consistent with our previous findings in other coastal countries such as El Salvador and Sri Lanka. Given recent literature that also suggests an interaction between rainfall and SH to predict influenza, further analyses in Kenya will also investigate this.

## 466

### INCIDENCE AND CHARACTERISTICS OF PATIENTS HOSPITALIZED WITH INFLUENZA INFECTIONS FROM A RESPIRATORY DISEASE SURVEILLANCE SYSTEM, GUATEMALA, 2008-2012

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Influenza is a major cause of respiratory illness affecting 5-15% persons worldwide, and resulting in 3-5 million severe cases and 250,000-500,000 deaths each year. Influenza-related hospitalizations cause substantial health and financial burden. We used an active facility-based surveillance system for acute respiratory disease in three hospitals in Guatemala to estimate the incidence of laboratory-confirmed hospitalized influenza cases (adjusted for healthcare seeking behaviors), characterize the cases, and identify risk factors associated with admission to the intensive care unit (ICU) or death. Laboratory confirmation was by real-time reverse transcriptase polymerase chain reaction. Multivariable logistic regression was used to identify risk factors for ICU admission or death, adjusted for age and sex. From May 2008 to July 2012 we identified 446 hospitalized influenza patients, 362 (81%) had influenza A and 84 (18%) had influenza B. The median age of case-patients was 2.4 years (interquartile range: 0.7-32.3). Median length of hospitalization was 5 days (range: 0-77). Eighty (17.9%) were admitted to the ICU, 28 (6.2%) died; overall, 88 (19.7%) experienced either ICU admission or death. Children aged <6 months comprised 19% of cases, 22% of those admitted to the ICU, and 7% of the deaths. Other deaths occurred in 11 (6%) children aged 7-60 months, 6 (6%) persons aged 5-50 years, and 9 (11%) patients aged ≥50 years. Women of child-bearing age comprised 6% of cases (2 admitted to ICU; 1 death). In multivariable analyses, being from Santa Rosa surveillance site, being of Amerindian ethnicity, and having radiologically-confirmed pneumonia were independently associated with ICU admission or death. The annual incidence of hospitalized laboratory-confirmed influenza in Santa Rosa and Quetzaltenango was 19.7/100,000 overall and 85.4/100,000 for children aged <5 years. Influenza is a major contributor of hospitalization due to respiratory diseases in Guatemala. Our findings warrant further investigations to explore the utility of enhanced influenza prevention strategies.

## 467

### USE OF THE FILMARRAY RESPIRATORY PANEL IN A RESOURCE-LIMITED POPULATION BASED COHORT-SETTING TO DETERMINE THE ETIOLOGY OF INFLUENZA-LIKE ILLNESS

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Influenza-like illness (ILI) can be caused by many pathogens that cannot be distinguished clinically. Conventional lab methods can be time consuming, labor intensive and only identify a single pathogen. The lack of an identifiable etiology can lead to empirical treatment of ILI due to lack of timely information. The FilmArray Respiratory Panel (RP) addresses many of these issues by providing an on-site, rapid testing methodology for 18 bacterial or viral respiratory pathogens. In August 2012, we implemented the FilmArray RP at our Puerto Maldonado, Peru satellite lab for use in an active household ILI surveillance study. During 33 weeks of surveillance, 413 ILI episodes were identified (7.9 episodes/1000 person-week of follow-up). The median age of ILI cases was 5 years old (IQR 12.7; SD 15.9). Samples were collected from 93.2% (385/413) of the ILI cases. The FilmArray RP identified at least one respiratory pathogen in 76.1% (271/356) of samples. A single pathogen was identified in 65.7% (234/356) of samples and multiple pathogens were identified in 10.4% (37/356) of samples. Among samples with a single etiology identified, human rhinovirus/enterovirus was identified in 29.9% (70/234) of samples; followed by influenza A H3, 23.1% (54/234); influenza A H1-2009, 11.5% (27/234); human metapneumovirus, 7.7% (18/234); respiratory syncytial virus (5.6%); and coronavirus OC43, 4.7% (11/234). The most common co-infection was human rhinovirus/enterovirus + parainfluenza virus 3 (29.4%,10/34). Three ILI (0.7%) required hospital admission; the etiologies identified were influenza A H1-2009, human metapneumovirus, and an influenza A H3 + human metapenumovirus co-infection. Numerous pathogens cause an ILI syndrome and influenza A was the predominant pathogen in our study. However, the FilmArray RP allowed us to determine the breadth of pathogens causing ILI and provide the opportunity for additional studies. Furthermore, on-site use of the FilmArray reduced time to results and reduced labor, shipping and lab testing costs.

## 468

### GLOBAL POPULATION STRUCTURE AND GENETIC EVIDENCE OF SELECTION OF SULFA RESISTANCE IN *PNEUMOCYSTIS JIROVECII*

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*Pneumocystis jirovecii* is a commensal respiratory pathogen that causes pneumonia (PcP) in immunosuppressed patients, principally those with advanced HIV infection. Because *P. jirovecii* cannot be cultured, it has proven difficult to study, and little is known about its global population structure and drug resistance profile. Using sequence from a *P. jirovecii* draft genome, we developed assays for 8 putatively neutral microsatellite *loci* and 1 locus adjacent to the dihydropteroate synthase (*dhps*) gene, in which mutations are thought to be associated with exposure to sulfa drugs and potential sulfa drug resistance. Using these assays, we genotyped isolates from Uganda (n=13), the United States (n=26) and Spain (n=29), investigated their population structure, and quantified evidence for selective pressure on mutant *dhps* haplotypes. In all three populations, the 8 neutral markers demonstrated high levels of heterozygosity ($H_e$ = 0.586 - 0.841). Although Nei's genetic distance indicated an overall genetic relatedness between populations (Uganda-Spain: 0.152; Uganda-US: 0.068; US-Spain: 0.034), when analyzed by an ecological clustering algorithm specimens from the three geographical populations demonstrated significant divergence of populations by continent. Notably, the single *dhps*-linked microsatellite marker demonstrated substantially lower diversity in isolates bearing mutant *dhps* haplotypes ($H_e$=0.305) compared with those bearing wildtype *dhps* haplotypes ($H_e$=0.682). This reduction in allelic diversity suggests that mutant *dhps* haplotypes have evolved and spread owing to selective pressure, most likely the exposure to sulfa drugs that are used for prevention and treatment of PcP as well as a wide variety of other bacterial and parasitic infections. The microsatellite-based multilocus genotyping method presented here represents the first genotyping tool to use selectively neutral *loci* for *P. jirovecii*, and will enable molecular-epidemiological investigations into *Pneumocystis* population structure, transmission, carriage, and drug resistance in human populations.

## 469

### THE ORGANIZATION AND EVOLUTION OF PIRNA CLUSTERS IN *ANOPHELES GAMBIAE*

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The Piwi-interacting RNA (piRNA) pathway is an important mechanism in the defense against transposable element (TE) mobilization in many species including *Drosophila melanogaster* (the fruit fly), *Aedes aegypti* (the Yellow Fever mosquito), and *Mus musculus* (the mouse). In *Drosophila*, it has been shown that clusters of piRNA produce transcripts that, when interacting with PIWI proteins, create a complex that can recognize and silence TEs in the germ-line. In *Aedes* mosquitoes and the mouse, piRNA clusters appear to involve a higher number of protein coding genes. The aims of our study were 1) to see if the piRNA mechanism in *Anopheles gambiae* is more closely related to the *Drosophila* pathway or to the *Aedes* pathway and 2) to test if incipient species of *An. gambiae* differ in the structure of piRNA clusters. Here, we show the results of piRNA sequencing of the M and S forms of *Anopheles gambiae s.s.* A database of uniquely mapping piRNAs has been developed and subsequently mapped to the chromosomal map developed for the PEST reference genome, a mixture of genomic sequences of the M and S forms. We have also identified clusters of piRNAs based on the mapping performed on the PEST genome. The largest clusters of piRNAs were, as expected, found primarily in high TE-content areas- the intercalary and peri-centromeric heterochromatin. The top 15 clusters identified in *Anopheles gambiae* could potentially produce ~74% of the total number of piRNAs. Our results demonstrate that piRNAs, much like in *Aedes* and *Drosophila*, are present and active in Anopheline mosquitoes, but differ between species. Divergence in piRNA sequences and cluster composition suggests that the defense mechanism in the two species has begun to rapidly evolve to protect its respective genome against novel TE invaders that are not accessible to both populations. Cluster locations and content also suggest that the piRNA pathway in Anopheline mosquitoes is an intermediary between the *Drosophila* and *Aedes* pathways, as the piRNA clusters appear to contain a higher quantity of TEs than is seen in *Aedes*. Our data suggests that although speciation between the M and S forms is recent, there are differences in the TE vestiges that make up the clusters, as well as the piRNA sequences that are both present and absent when mapped to their respective genomes.

## 470

### CRE-RECOMBINASE MEDIATED CASSETTE EXCHANGE IN *AEDES AEGYPTI*

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Approaches to generate transgenic mosquitoes rely on the random incorporation of a transposable element or site-specific integration utilizing a previously characterized targeting site. To date, the only site-specific technique currently used in disease-vector mosquitoes is the phiC31 system. Efficiency of recombination using this system varies extensively by target site and successful recombination results in the simultaneous integration of the entire donor plasmid. An alternative system based on cre (cause recombination) recombinase has been shown to be highly effective in mosquitoes, but strongly favors excision over integration. Recombinase-mediated cassette exchange (RMCE) involves the site-specific exchange of DNA flanked by sequences that are substrate for a particular recombinase. In particular, heterospecific lox sites resist in cis recombination (excision). We have developed two *in vivo* quantitative plasmid embryo assays to assess RMCE potential in *Aedes aegypti* using heterospecific pairs of lox sites (locus of crossing-over). *Ae. aegypti* embryos were injected with plasmids designed to quantitatively determine the comparative excision rate of cre upon heterospecific lox sites flanking firefly luciferase ORF. Three candidates exhibited relatively low excision in the presence of cre, these particular sites were then further evaluated for their RMCE potential. All three lox sites tested demonstrated significant RMCE within the embryo inter-plasmid assay. We are currently generating stable transgenic lines that will allow us to determine RMCE potential between a plasmid donor and the different heterospecific lox sites of the multi-functional target cassette. Our end goal is to develop a tool for the transgenic field that will be used to further investigate biological pathways within mosquitoes.

## 471

### INCREASED LIGHT SENSITIVITY UNDER LOW-LIGHT CONDITIONS ASSOCIATED WITH EXTENSIVE AAOP1 REDISTRIBUTION IN *AEDES AEGYPTI*

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We are evaluating the role of vision and circadian cues in the activity and behavior of the disease vector *Aedes aegypti*. At night, a time period for which *Ae. aegypti* is not typically active, low levels of light exposure triggered robust mosquito activity, indicating that light acts outside the realm of circadian input to influence mosquito behavior. The *Ae. aegypti* compound eye is composed of approximately 300 ommatidia, each containing a bundle of eight (R1-R8) photoreceptor cells. The blue light-sensitive Aaop1 is a major rhodopsin of the retina, being expressed in the R1-6 photoreceptors cells. The photosensitive organelle in these cells, known as the rhabdomere, has a microvillar structure that allows a large plasma membrane surface to concentrate rhodopsins and other proteins that function in phototransduction. The Aaop1 rhodopsin is localized to vesicles in the cytoplasm during daylight and absent from the photosensitive membranes of the rhabdomere. At dusk, Aaop1 moves from cytoplasmic multi-vesicular bodies and becomes localized within the rhabdomeres. We examined the influence of circadian rhythms on Aaop1 behavior by exposing the animals to sustained light or precocious dusk. These experiments showed that light itself, not circadian signals, is responsible for the movement of Aaop1. We also carried out protein blot analysis to examine the levels of Aaop1 at different times in the day/night cycle. The results showed that Aaop1 levels gradually increase during the morning period but then declines during the afternoon and evening periods. It is likely that a circadian input influences the change in Aaop1 levels because the decrease was still observed even when light cycles were altered. We also examined the effect of rhodopsin translocation on light sensitivity. There is a correlation between Aaop1 translocation and increased light sensitivity when measured by electroretinogram. The results show there is approximately a 1.5 log unit increase in sensitivity upon translocation of Aaop1 into the rhabdomeres, establishing a correlation between Aaop1 translocation at dusk and increased light sensitivity.

## 472

### THE GENETIC BASIS OF HUMAN HOST CHOICE IN THE MALARIA VECTOR *ANOPHELES GAMBIAE*

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The dominant African malaria vector *Anopheles gambiae* s.s preferentially takes it blood meals from human hosts, often at rates as high as 90% in natural populations. Its adaptation to human hosts has a genetic basis in the olfaction system, which includes several key gene families - the olfacation receptors (ORs), ionotropic receptors (IRs), odorant binding proteins (OBPs). To identify *An. gambiae* genes responsible for human host preference, we conducted a quantitative trait *loci* (QTL) mapping experiment based on backcrosses between the anthropophilic *An. gambiae* and the zoophilic An. quadriannulatus. Backcross females were subjected to a host-choice experiment in an olfactometer in which they were presented with a human and cow odor. Only individuals that selected the same odor on three consecutive days were included in the experiment. A total of ~15,000 individual backcross females were run through host-choice experiments, resulting in two pools totaling 432 mosquitoes with divergent host preferences. We used 38 microsatellite markers to genotype individuals and performed Multiple Interval Mapping using R/QTL. We identified six narrow QTL for human host preference on chromosomes 2 and 3, that together explain 49% of the phenotypic variance. The X chromosome did not contribute significantly to human host preference. A total of 34 ORs, 7 IRs and 21 OBPs are located inside QTL, but three QTL contain only between 4-6 olfaction genes. In addition, a comparison of antennal transcriptomes identified 13 olfaction genes that are located inside QTL and that were significantly higher expressed in *An. gambiae* vs *An. quadriannulatus.*

## 473

### MALE ACCESSORY GLAND FUNCTION IN THE DENGUE VECTOR, *AEDES AEGYPTI*

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Seminal fluid proteins (SFPs) are produced in the reproductive tract of male insects and are transferred, along with sperm, to females during mating. SFPs induce physiological and behavioral changes in the mated female. In the dengue vector mosquito, *Aedes aegypti*, seminal fluid stimulates egg production , induces mating refractoriness, and increases survival rate when injected into females. To date, we have identified 93 male-derived proteins. To learn more about how the male AG functions and how SFPs are produced and secreted, we are studying a novel SFP, AAEL010824. We show that 10824 protein production is specific to the male accessory gland tissue. In addition, 10824 is fully depleted in males after 5 succesive matings , but is almost completely replenished after 48h. To further define 10824's expression pattern, we used 10824 promoter sequences to drive expression of a reporter construct (GFP). Expression is found exclusively in the anterior cells of male accesory glands, suggesting that the gland is composed of more than one cell-type. These results will aid future work to characterize SFP production and secretion in *Ae. aegypti*.

## 474

### TALEN-BASED GENE DISRUPTION IN THE DENGUE VECTOR *AEDES AEGYPTI*

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*Aedes aegypti* is the primary vector for dengue viruses (serotypes1-4) and chikungunya virus. The inability to control this vector, and thus the disease agents it transmits, has prompted the development of novel genetic-based control strategies. TALE nucleases (TALENs) have been used with great success in a number of organisms to generate site-specific DNA lesions. We evaluated the ability of a TALEN pair to target the *Ae. aegypti* kmo gene, whose protein product is essential in the production of eye pigmentation and confirmed that TALEN-based gene disruption can be a highly efficient process in *Ae. aegypti*, with editing rates between 20-40% which is greater than both traditional transposon-based transformation and phiC31-based recombination. Mutant alleles were associated with lesions of 1-7 bp specifically at the selected target site. White-eyed individuals could also be recovered following a blind intercross of G1 progeny, yielding several new white-eyed strains in the genetic background of the sequenced Liverpool strain. We compared the fitness of these new white-eyed strains in terms of the time of bloodfeeding, larval viability, fertility as a function of egg hatchability and adult mosquito survivorship. We have also investigated the rate of homologous recombination stimulated by TALEN activity by introduction of a green fluorescent protein (GFP) reporter gene into the *Ae. aegypti* germline. We conclude that TALENs are highly active in the early embryos and germline of *Ae. aegypti* mosquitoes, and have the potential to transform how reverse genetic experiments are performed in this important disease vector.

## 475

### BIOCHEMICAL AND MOLECULAR STUDIES OF ALANINE AMINOTRANSFERASE IN *AEDES AEGYPTI* MOSQUITOES

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Alanine aminotransferase (ALAT, EC 2.6.1.2) participates actively in maintaining the alanine-proline cycle between flight muscles and fat body when proline is utilized as an energy substrate during flight in blood-fed *Aedes aegypti* mosquitoes. Additionally, ALAT participates in the ammonia metabolism when *A. aegypti* females are fed a blood or ammonia meal. In order to better understand the mechanisms underlying the ALAT activity in mosquito metabolism, we are currently using multiple approaches. First, we investigated how *A. aegypti* females respond to blood meals supplemented with 0, 2.5, 5 and 10 mM of L-cycloserine, a well-known inhibitor of ALAT in animals. In some experiments, females were starved and then fed a blood meal containing L-cycloserine in the presence or absence of glucose. Survival and motor activity were recorded during a time course (1, 2, 4, 6, 12, 24, 48 and 72 h after feeding). L-cycloserine at 10 mM resulted in high mortality relative to control, with an acute effect during the first 6 h after treatment. A significant reduction of motor activity and blood meal digestion, as well as an increase in futile wing vibration during the first 24 h was observed at all inhibitor concentrations. The starvation and supplementation of glucose in the diet amplified the effect of L-cycloserine. Next, we analyzed the expression pattern of two genes encoding ALAT (1 and 2) in sugar or blood-fed *A. aegypti* tissues. The data show a distinct expression pattern for each gene in mosquito tissues collected at different times after feeding (0-120 h). Finally, we injected dsRNA against ALAT 1 or ALAT 2 into newly-emerged females. In agreement with the results described above, the silencing of either gene by RNAi causes a significant retention of the blood meal, a significant reduction of the nitrogen waste excreted, and an unexpected and dramatic accumulation of nitrogen waste in the midgut. Our studies indicate that *A. aegypti* ALAT plays a critical role in blood-fed *A. aegypti* nitrogen metabolism.

## 476

### *YERSINIA* MURINE TOXIN IS NOT REQUIRED FOR EARLY-PHASE TRANSMISSION OF *YERSINIA PESTIS* BY *OROPSYLLA MONTANA (SIPHONAPTERA: CERATOPHYLLIDAE)*

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Plague, caused by *Yersinia pestis*, is characterized by quiescent periods punctuated by rapidly spreading epizootics. The classical "blocked flea" paradigm, by which a blockage forms in the flea's proventriculus on average 1-2 weeks post infection, forces starving fleas to take multiple bloodmeals, thus increasing opportunities for transmission and is undoubtedly significant during inter-epizootic periods. Recently the importance of early-phase transmission (EPT) during epizootics has been emphasized. EPT occurs prior to blockage formation and fleas are immediately infectious. While the physiological and molecular mechanisms of transmission are well characterized for the blocked flea model, the pathogen-vector interactions have not been defined for EPT. Within the blocked flea model, two genes, *Yersinia* murine toxin (*ymt*) and the hemin storage locus (*hms*), have been shown to be important for facilitating colonization of the midgut and proventricular blockage within the flea. One proposed mechanism of EPT is the regurgitation of infectious material from the flea's midgut during feeding. Such a mechanism would require bacteria to colonize the midgut, a process that is mediated by *ymt*. *Oropsylla montana*, an important bridging vector in North America, was used in our study to test this hypothesis. Fleas were infected with a mutant strain of *Y. pestis* containing a nonfunctional *ymt* incapable of colonizing the midgut; infected fleas were allowed to feed on SKH-1 mice 3 d post infection. Our results show that colonization of the flea midgut by *Y. pestis* is not required for EPT. Furthermore, our results, in combination with a previous study showing that *hms* is not required for EPT, clearly demonstrate that the vector-pathogen interactions that define EPT are distinct from those in the blocked flea model.

## 477

### HOST CUTANEOUS RESPONSES TO THE ROCKY MOUNTAIN SPOTTED FEVER VECTOR, *DERMACENTOR ANDERSONI,* FEEDING

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Tick salivary glands produce complex cocktails of bioactive molecules that facilitate blood feeding and pathogen transmission by modulating host hemostasis, pain/itch responses, wound healing and both innate and adaptive immunity. In this study, cutaneous responses at Dermacentor andersoni bite-sites were analyzed using Affymetrix mouse genome arrays and histopathology at 12, 48, 96 and 120 hours post infestation (hpi). The microarray data suggests: (1) chemotaxis of neutrophils, monocytes, and other cell types; (2) production and scavenging of reactive oxygen species; (3) keratin based wound healing responses. The histological analysis supported the microarray data findings. At 12hpi, a mild inflammatory infiltrate was present in the dermis, especially concentrated at the junction between dermal connective tissue and underlying adipose tissue. A small lesion was located immediately under the hypostome and likely represents the feeding "pool." Surprisingly, at 48hpi, the number of inflammatory cells had not increased from 12hpi, perhaps mirroring the reduction in gene expression seen at this time point. The feeding lesion is very well defined, and extravasated erythrocytes are readily evident around the hypostome. By 96hpi, the inflammatory infiltrate has increased

dramatically and the feeding lesion appears to have moved deeper into the dermis. At 120hpi, most of the changes at 96hpi are intensified. The infiltrate is very dense, the epidermis is markedly thickened, the feeding lesion is poorly defined and the dermal tissue near the hypostome appears to be loosing its normal architecture. In conclusion, during *Dermacentor andersoni* feeding infiltration of inflammatory cells increases across time concurrent with significant changes in the epidermal and dermal compartments near the feeding tick. The importance of changes in the epidermal layer in the host response to ticks is not known, however, it is possible the host attempts to "slough off" the tick by greatly increasing epithelial cell replication.

## 478

### HUMAN BEHAVIORAL AND ECOLOGICAL RISK FACTORS FOR LYME DISEASE INFECTION ON BLOCK ISLAND, RHODE ISLAND

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Peridomestic exposure to infected *Ixodes scapularis* nymphs is considered the dominant means of infection with tick-borne pathogens in the eastern United States. Previous studies of risk of developing tick-borne infection established a positive association between the density of infected nymphs and Lyme disease cases at the population level. Studies examining the effectiveness of personal protective behaviors have not included measures of tick exposure. This study simultaneously assesses the effect of tick exposure and human behavior in Lyme disease infection risk using a longitudinal serosurvey study on Block Island, Rhode Island. Tick exposure risk at all Island properties was estimated by identifying remotely-sensed landscape proxies that most strongly correlated with tick density at the individual property level. Landscape metrics associated with lawn and shrub edge, density of shrub patches and percent of the landscape occupied by shrubs and the number of patches were found to be most strongly associated with positive serology. Human behavior related risk factors included the average number of hours spent daily outside in tick habitat, and owning a cat that spends time both indoors and outdoors. Age at the time of test was also found to increase risk. Wearing protective clothing during outdoor exposure was protective. A multivariate model including peridomestic shrub patch density (decreased risk), wearing protective clothing (decreased risk), and owning a cat (increased risk) was determined to be the best model based on the lowest Akaike Information Criterion. Our findings emphasize that both environmental risk and human behavior contribute significantly to risk of tick-borne infection. They highlight the importance of accounting for environmental exposure to accurately ascertain the effectiveness of personal protective behaviors. A better understanding of the relative roles of environmental and behavioral risk factors in driving infection with tick-borne pathogens should guide future intervention studies to reduce the risk of these infections.

## 479

### THE INFLUENCE OF LAND COVER, ENVIRONMENTAL AND SOCIO-ECONOMIC FACTORS ON THE SPATIAL DISTRIBUTION OF SCRUB TYPHUS IN TAIWAN

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Environmental factors, including land cover and land use, are known to influence breeding and survival of trombiculid mites and, thus, the spatial heterogeneity of scrub typhus risk, which is transmitted by the mites' larval stage. Here, a spatially autoregressive modelling framework was applied to scrub typhus incidence data from Taiwan, covering the period 2003 to 2011, to provide improved understanding of the spatial pattern of scrub typhus risk and the environmental and socio-economic factors contributing to this pattern. A clear spatial pattern in scrub typhus incidence was observed within Taiwan, and incidence was found to be significantly correlated with several land cover classes, temperature, elevation, NDVI, rainfall, population density, average income and the proportion of the population that work in agriculture. The final multivariate regression model included statistically significant correlations between scrub typhus incidence and average income (negatively correlated), the proportion of land which contained mosaics of cropland and vegetation (positively correlated) and elevation (positively correlated). These results highlight the importance of land cover on scrub typhus incidence: mosaics of cropland and vegetation represent a transitional land cover type which can provide favourable habitats for rodents and, therefore, trombiculid mites. In Taiwan, these transitional land cover areas tend to occur in less populated and mountainous areas, following the establishment and subsequent partial abandonment of agricultural cultivation, due to demographic and socio-economic changes. Future land use policy decision making should ensure that potential public health outcomes, such as modified risk of scrub typhus, are considered.

## 480

### LYME DISEASE RISK IN A SPECIES-POOR ISLAND: LACK OF EVIDENCE FOR THE DILUTION EFFECT

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Biodiversity's role in buffering against human infectious disease is proposed as an ecosystem service applicable to many diseases. The dilution effect hypothesizes that increased host diversity reduces pathogen transmission by reducing direct or indirect contact between hosts. Biodiversity may also influence disease risk if pathogens can specialize to different host species. Lyme disease, caused by the bacterium *Borrelia burgdorferi* and transmitted by *Ixodes scapularis* ticks, is a common zoonotic disease in the northeastern United States. The dilution effect predicts that host communities dominated by *Peromyscus leucopus*, the primary reservoir host, represent the highest risk to humans. Under the host specialization hypothesis, reduced biodiversity may increase disease risk because some strains can persist longer in *P. leucopus* and are more likely to be transmitted to larvae. In a comparative study of a host species-poor community dominated by *P. leucopus* and a host species-rich community, we evaluated support for two specific predictions of these hypotheses: 1) *B. burgdorferi* nymphal infection prevalence and density of infected nymphs is higher in the species-poor than species-rich community, and 2) *B. burgdorferi* genotype diversity is higher in the species-rich than the species-poor community. We collected mammal-derived larvae and questing nymphs in 2010 and 2011 to measure infection prevalence and estimate genotype diversity and richness. We found no differences in nymphal infection prevalence and density of infected nymphs between the two communities, providing no evidence for the dilution effect. We also found high levels of genetic variation in the species-poor community, refuting the host specialization hypothesis. Estimates of genotype diversity and richness of disseminating *B. burgdorferi* strains found primarily in *P. leucopus* were not significantly different between species-poor and species-rich communities. We find little evidence for either hypothesis, suggesting a complicated relationship between biodiversity and disease prevalence.

## 481

### SCABIES PREVALENCE IN KONGWA DISTRICT, TANZANIA

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Scabies is one of the most common communicable diseases in the world, with prevalence as high as 20% in some endemic, resource-poor settings. The mite *Scabies sarcoptii* burrows in the skin, causing itching, and these sores can easily become super-infected with bacteria. Scabies responds to treatment with ivermectin, and scabies cases have been reported to decrease in areas receiving mass drug administration (MDA) with ivermectin, but to date this has only been examined in retrospective studies. We are therefore undertaking a four-year prospective study of scabies prevalence in eight villages in Kongwa District, Dodoma Region, Tanzania, where MDA with ivermectin is being given for lymphatic filariasis. Community-wide baseline surveys were done on all ages in October-December of 2012 by clinical examination of extremities by a trained community health worker. A total of 2269 individuals in 8 villages were examined; of these, most were between the ages of 1-9 (1631, 71.9%). The overall scabies prevalence was 4.3% (97/2269), with most scabies cases being detected in individuals <15 years of age (89 cases in 1-9 year olds, 6 cases in 10-15 year olds). Only 2 cases were seen in individuals >15 years old. Forty-six cases of infected scabies were observed, and skin swabs of scabies sores from all clinically-positive individuals are currently being tested for the presence of bacterial superinfection. Itching was reported in all clinical scabies cases. Scabies prevalence in 7 villages ranged from 2.9 - 5.7%, while one village had a particularly high prevalence of 14.0%. These villages will be followed over the next three years of ivermectin treatment to determine if scabies prevalence drops as a collateral benefit of the lymphatic filariasis MDA program.

## 482

### EPIDEMIOLOGICAL AND GEOGRAPHIC DISTRIBUTION OF TICK-BORNE RELAPSING FEVER BORRELIOSIS IN WEST AND NORTH AFRICA

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Tick-borne relapsing fever (TBRF) is often confused with malaria in Africa and the epidemiology and geographic distribution of the disease are poorly known. Our aim was to investigate the distribution of the vectors and reservoirs of *Borrelia* infections in West and North Africa. From 2002 to 2012, we conducted field surveys in 17 African countries and in Spain. We investigated the occurrence of *Ornithodoros* ticks in rodent burrows in 282 study sites. We collected 1,629 small mammals that may act as reservoir for *Borrelia* infections. Using molecular methods we studied genetic diversity among *Ornithodoros* ticks and *Borrelia* infections in ticks, small mammals and patients with relapsing fever. Of 9,870 burrows investigated, 1,196 (12.1%) were inhabited by *Ornithodoros* ticks morphologically attributable to *O. sonrai, O. erraticus* or *O. normandi*. We collected these ticks in West Africa then North (including Western Sahara) and Spain. In West Africa, the southern and eastern limits of the vector and *Borrelia* infections in ticks and small mammals were 13°N and 01°E, respectively. Molecular studies revealed the occurrence of nine different *Ornithodoros* species, with six of them harboring *Borrelia* infections. The distribution of *O. erraticus* stricto sensu appeared restricted to wet coastal areas of eastern Algeria and western Tunisia and its role as TBRF potential vector was not confirmed. Only *B. crocidurae* was found in West Africa and three - 3 - *Borrelia* species were identified in North Africa: *B. crocidurae, B. hispanica*, and *B. merionesi*. *Borrelia* spirochaetes responsible for TBRF in humans are highly prevalent both in *Ornithodoros* ticks and small mammals in North Africa and northwestern West Africa but *Ornithodoros* ticks seems absent from other regions of West/Central Africa and small mammals are not infected in these regions. Genetic diversity among TBRF *Ornithodoros* vectors is much higher than previously known. Unknown vector / reservoir systems of *Borrelia* infections may exist in wet savanna areas of West Africa.

## 483

### IMMUNE RESPONSES TO CERCARIAL ANTIGENS IN HUMAN POPULATIONS ENDEMICALLY EXPOSED TO SCHISTOSOME INFECTION

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Schistosomes are parasitic helminths which account for over 200,000 deaths per year in sub-Saharan Africa alone. People inhabiting schistosome-endemic regions are repeatedly exposed to infective schistosome larvae (cercariae), which actively penetrate the skin facilitated by the release of excretory/secretory (E/S) molecules. However, the immune response to cercariae and their E/S antigens remains poorly understood in humans. To address this gap in our understanding of schistosome immunobiology two studies were conducted in schistosome-endemic communities. In order to characterise cercariae-specific cytokine profiles, venous blood was collected from a *Schistosoma haematobium*-exposed community in Northern Zimbabwe (n=72) and then cultured with antigens from whole cercariae (cercarial antigen preparation, CAP) for 48 hours. Subsequently 13 CAP-specific cytokines were quantified by enzyme-linked immunosorbent assay (ELISA). This study identified CAP as a potent inducer of pro-inflammatory cytokines (IFN-γ, TNF-α, Interleukin (IL-) 6, IL-8, IL-12p70 and IL-23p19) As well as regulatory IL-10. Importantly these cytokines were further elevated 6 weeks after treatment with the anti-helminthic drug praziquantel (n=21). A subsequent study focused on immune responses to cercarial E/S antigens, which are released in the skin within the first 3 hours post-infection, in order to investigate whether E/S-specific immune responses could be detected in the peripheral blood of people inhabiting an *S. haematobium* and *S. mansoni* co-endemic region of Senegal. In this community venous blood cytokine responses were assayed by ELISA (n=47) and *in vitro* binding of peripheral blood mononuclear cells (PBMCs, n=41) to cercarial E/S antigens of *S. mansoni* was quantified via flow cytometry. Both IL-10 responses to E/S antigens and binding of CD14+CD16+ monocyte subsets to cercarial E/S antigens were greatest in schistosome-infected individuals relative to un-infected people. Together these studies shed light on the immune response to cercariae, the first stage of the schistosome life-cycle encountered by the human host.

## 484

### DETECTION OF MULTIFUNCTIONAL CYTOKINE-SECRETING B REGULATORY CELLS IN MALIAN CHILDREN WITH OR WITHOUT *SCHISTOSOMA HAEMATOBIUM* INFECTION

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The complex interplay between parasite and host induces an immunomodulatory response that may alter the usual host immunity. While B cells are known for antibody production, a subset of B cells produce cytokines, in particular IL-10, and appear to be antigen-activated with the ability to directly suppress effector T cell responses. These immunoregulatory B cells, termed Bregs, may play an important role in regulating parasitic infections but methods of detection are needed. We have optimized a flow cytometric panel that include monoclonal antibodies against CD19, CD3, CD38, CD27, CD24, CD1d, CD5, IgD, and intracellular cytokine detection of IL-10, IL-6, TNF-α, and IFN-γ. Peripheral blood mononuclear cells (PBMC) collected from age-matched Malian children with (SP; n = 5) or without *S. haematobium* (SN; n=5) infection were primed with anti-IgG/IgM antibody or CpG (ODN 2006) followed by a brief stimulation with PMA/ionomycin in the presence of Brefeldin-A. The phenotype and cytokine response of CpG-activated Bregs in SP and SN were of equal magnitude. The majority of the responding Bregs were single-positive for IL-10 (mean±SE: 37.4±1.8%); however double- and triple-positive subsets were also present [IL-10+IL-6+ (36.4±1.8), IL-10+TNF-α (7.7±1.4), and IL-10+IL6+TNF-α+ (17.9±2.4)]. Very few Bregs were found to express INF-γ. However, significantly higher levels of multifunctional Bregs were observed in PBMC from SP children compared to those observed in SN children in response to CpG. Moreover, preliminary results suggest that CpG in conjunction with schistosoma soluble egg antigen (SEA) elicited a 2 to 4 fold higher proportion of Bregs co-expressing IL-6 and IL-10 in SP children. These studies, using an optimized method for the detection of Bregs demonstrate, for the first time, multifunctionality in this B cell subset and heterogeneity in their phenotype. Moreover, they suggest that Bregs in SP children may have higher responsiveness to CpG and CpG + SEA than in SN subjects. The potential role of Bregs in parasitic immunomodulation requires additional study.

## 485

### *IN VITRO*, HUMAN EOSINOPHILS DOWN MODULATE PERIPHERAL BLOOD MONONUCLEAR CELLS RESPONSES TO *SCHISTOSOMA MANSONI* ADULT WORM ANTIGENS

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Eosinophils have been regarded as terminally differentiated non-replicating effector cells observed in a number of health disorders including parasitic infections and allergic diseases where they play a beneficial role in the host defense against helminth infections or cause a harmful inflammatory response respectively. There is growing evidence that eosinophils can play an additional immunoregulatory role in both adaptive and innate immunity to parasitic infections. We investigated the effects of human eosinophils on peripheral blood mononuclear cells (PBMC) response to *Schistosoma mansoni* adult worm antigen. we have observed that when PBMCs obtained from *S. mansoni* infected adults were examined for cytokine responses to *S. mansoni* adult worm antigen (SWA), eosinophils exerted a down-modulatory effect on schistosome specific responses. The mechanism of this immune-modulation remains to be elucidated.

## 486

### ASSOCIATION OF MODULATORY CYTOKINE GENE POLYMORPHISMS IN RESISTANCE AND SUSCEPTIBILITY TO POLYPARASITISM INFECTION IN ZIMBABWE

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Single nucleotide polymorphisms within the cytokine genes, TNF-α (-308 G/A), IFN-γ (+874 A/T), TGF-β (T/C codon 10 and G/C codon 25) and IL-10 (-1082 G/A and -819 T/C) associated with moderation or severity of parasitic infections were examined in samples from school children aged between 5 - 16 years. About 72.8% were infected with either malaria and/or different helminths. Genotyping was carried out using the ARMS-PCR method. The frequency of TNF-α (GG) associated with low cytokine production was 76.1%, while 22.2% and 1.6% were predictors of medium and high production of TNF-α, respectively. For IFN-γ (+874 A/T), 70.5% were (AA) associated with high cytokine secretion, with 4.4% (TT) and 25.1% (AT) associated with low cytokine production. Limited analysis on the samples also revealed that at the TGF-β locus (T/C codon 10) 88.5% were TT, which predicts high production of the cytokine, whereas 9.2% were CC. Similar analysis at another locus of TGF-β (G/C codon 25) showed that only 2.3% showed GC predicting high TGF-β production. Equal distribution of IL-10 (-819 G/A) and the rare occurrence of allele associated with low IL-10 (-1082 AA) production would suggest moderate to high IL-10 responses in the population. Finally, the high prevalence of TGF-β genotype (TT) predicting high cytokine production and the existence of IL-10 (high producer) might suggest the dominance of an anti-inflammatory environment when faced with acute P.*falciparum* infection in the population. These observations may also suggest a complex interaction between various cytokine gene polymorphisms and high burden parasitic infections in the area.

## 487

### SCHISTOSOMIASIS DURING PREGNANCY IS ASSOCIATED WITH IMPAIRED VACCINE EFFICACY IN KENYAN INFANTS

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Maternal parasitic infections during pregnancy prime the fetal immune response and induce an immunomodulatory phenotype at birth that may affect subsequent immune responses to childhood vaccines. This study determined whether urinary schistosomiasis in pregnant women influenced antibody levels to *Haemophilus influenzae* type B (Hib), hepatitis B (Hep B), diphtheria toxoid (DT) and tetanus toxoid (TT) following vaccination in their offspring. 480 Kenyan women were tested for urinary schistosomiasis (schisto), malaria, lymphatic filariasis and intestinal helminthes during pregnancy. Plasma samples were collected from their offspring every 6 months from birth to 3 years of age and IgG antibody levels to Hib, Hep B, diphtheria and TT vaccinations were measured by ELISA. Although 64% of the pregnant women were infected with one or more parasites, urinary schistosomiasis (30%) was one of the most prevalent infection with consistently detectable lymphocyte responses to *S. hematobium* soluble worm extract (SWAP) as determined by Th2/Th1 cytokine production in cord blood (sensitized, N=239). Other children were born to schistosomiasis infected mothers, but lack cord

blood recall responses to SWAP (putatively immune tolerant, N=45). Some children were born to mothers without schistosomiasis (unexposed, N=91). Using a linear mixed effects models adjusted for maternal age and parity, children characterized with an immunotolerant phenotype due to schisto-driven fetal priming had a lowest vaccine-induced antibody response to Hep B (P=0.001, 0.001, 0.002 and 0.01), DT (P=0.003 and 0.01), and TT-specific IgG (P=0.01, 0.03 and 0.019) at 6, 12, 18 and 30 months of age compared to unexposed children. Thus, urinary schistosomiasis during pregnancy may impair childhood vaccine efficacy. This data highlights the importance of programs to eradicate parasitic infections in pregnant women.

## 488

### LONGITUDINAL ANALYSIS OF ANTIGEN SPECIFIC RESPONSE IN INDIVIDUALS WITH *SCHISTOSOMA MANSONI* INFECTION IN ENDEMIC AREA OF MINAS GERAIS

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Immunoepidemiologic studies have established a clear relationship between IgE and IgG4 antibodies with age, resistance to infection, susceptibility and prediction of infection. It is believed that IgE and IgG4 response to soluble egg antigen (SEA) can be used as a biomarker for infection by *Schistosoma mansoni*, i.e use serological evaluation as basic tool for faster analysis of infection. The purpose of this investigation was to evaluate whether anti-SEA IgE and IgG4 reactivity can be useful as a biomarker to assist monitoring of infection with *S. mansoni*. Between 2001 and 2009, a longitudinal study was performed in Virgem das Graças, Brazil. Parasitological and blood specimens were collected from 127 individuals. Patient sera was tested by ELISA for anti-SEA IgE and IgG4. The schistosomiasis prevalence and geometric mean egg count in 2001 was 59% and 61.05%, respectively and decreased in the following years reaching 26.8% and 8.78% in 2009. The IgG4 anti-SEA reactivity in infected individuals was significantly higher than of uninfected in investigated periods. Analysis of ROC area showed that the IgG4 anti-SEA antibodies were able to predict infection by *S. mansoni* in all study years. Our results showed that the IgG4anti-SEA reactivity can be used as a biomarker for immune monitoring to predict infection outcomes with *S. mansoni* in endemic areas.

## 489

### PROPORTIONS OF CD4+ BUT NOT CD8+ MEMORY T CELLS ARE ALTERED IN OLDER INDIVIDUALS CHRONICALLY INFECTED WITH *SCHISTOSOMA HAEMATOBIUM*

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Protective immunity against human helminth infection develops slowly. Characterisation of parasite-specific responses has focused on effector responses with little work conducted on memory responses in human or experimental studies. Effective generation and maintenance of memory immune responses are central to the development of protective immunity against re-infection and for successful vaccinations. Here we show for the first time, that human helminth infection is associated with altered proportions of the CD4+ memory T cells, with an associated alteration of TH1 responses. Helminth infection does not affect the CD8+ memory T cell pool. Furthermore, we show for the first time in a helminth infection that the CD4+ memory T cell proportions decline following curative anti-helminthic treatment despite increased CD4+ memory cell replication. Reduced accumulation of the CD4+ memory T cells in schistosome-infected people has implications for vaccination programs and may contribute to the reduced vaccine efficacy already reported in helminth infected populations.

## 490

### MULTIPLEX REAL-TIME PCR DEMONSTRATES FOCAL DISTRIBUTION OF *STRONGYLOIDES STERCORALIS* IN DIFFERENT ENDEMIC COUNTRIES

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A major reason why *Strongyloides stercoralis* is highly neglected, even in studies aiming to examine the epidemiology and control of clinically relevant Soil Transmitted Helminths (STH), is the fact infections are simply missed in commonly used diagnostic procedures such as kato-katz stool slide examination. Specific detection and quantification of *Strongyloides* DNA by multiplex real-time PCR has recently been described as a sensitive and highly specific diagnostic method. In the present study we compare prevalence and intensity of *Strongyloide*s infection in different geographical regions using the a standardized real-time PCR procedure. In total more than 4.000 stool samples from both adults and children were collected in eight tropical countries across three different continents. All samples were collected in order to study the epidemiology of helminths within rural or semi-urban communities. DNA isolation, amplification and detection were performed using identical protocols. Results were obtained for *Ascaris lumbricoides*, *Necator americanus*, *Ancylostoma duodenale* and *S. stercoralis*, of which only the latter will be presented here. The percentage of *S. stercoralis* specific DNA stool positive individuals ranged from less than 1% in Senegal to 10% in Ghana and more than 40% in Mozambique and Peru. In those settings where extensive and *Strongyloides*-dedicated microscopy (i.e. Baermann and copro-culture procedures) was used, PCR-based findings confirmed the detection of L3-larvae. Our data shows a high variation in prevalence and intensity of *S. stercoralis* infection in different communities, ranging from almost zero to extremely high infection levels. In comparison to microscopy, multiplex real-time PCR was found to be a much more sensitive, more reliable and relatively simple high throughput procedure to monitor *Strongyloides* infections in cross sectional surveys. The use of multiplex real-time PCR for the detection and quantification of *Strongyloides* DNA allows for improved comparison of the epidemiology of this infection between different geographical regions.

## 491

### INSULIN/IGF-1-LIKE AND STEROID-NUCLEAR HORMONE SIGNALING ACT IN PARALLEL TO REGULATE DEVELOPMENTAL ACTIVATION OF THIRD-STAGE LARVAE OF *STRONGYLOIDES STERCORALIS*

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Dauer development by the free-living nematode *Caenorhabditis elegans* is considered a model for development by parasitic nematode larvae during host infection. In *C. elegans*, dauer arrest and recovery are regulated in part by insulin/IGF-1-like signaling (IIS) via a steroid-nuclear hormone receptor (NHR) pathway operating downstream. Evidence for ordering of these pathways includes the fact that mutations in the DAF-12 NHR suppress dauer constitutive (daf-c) mutations in signaling kinases in the IIS pathway. Administering a DAF-12 ligand, delta 7-dafachronic acid (DA), to *C. elegans* also suppresses some daf-c mutations, but only partially in the case of strong mutations in DAF-2. This suggests that to a degree, IIS operates parallel to, not upstream of NHR signaling in *C. elegans*. Dauer signaling pathways are conserved in the parasitic nematode *Strongyloides stercoralis*, and IIS is required for developmental arrest and activation of its infective third-stage larvae (L3i). We previously characterized an insulin-regulated PI3 kinase, *Ss*-AGE-1, and showed that its chemical inhibition prevents activation of *S. stercoralis* L3i under host-like culture conditions. Here we asked whether IIS in *S. stercoralis* also works in parallel to NHR signaling by ascertaining whether DA, which promotes activation of *S. stercoralis* L3i *in vitro*, can rescue inhibition of L3i activation by the PI3K inhibitor LY294002. We cultured L3i at 37C and 5% $CO_2$ in M9 buffer containing either 400 nM DA, which promotes maximal activation, or 400 nM DA plus 100 µM LY294002, which completely inhibits activation. Frequency of activation, indicated by the percentage of larvae ingesting FITC from the medium, was determined after 24 hours. As expected, 92.8% of DA-treated larvae ingested FITC. Significantly, only 8.6% of larvae exposed to both DA and LY294002 ingested FITC. This indicates that chemical inhibition of *Ss*-AGE-1 is not suppressed by administered DA and supports the hypothesis that IIS operates largely parallel to steroid NHR signaling to promote developmental activation of *S. stercoralis* L3i.

## 492

### THE MOLECULAR CHARACTERIZATION OF RIO PROTEIN KINASE-ENCODING GENES FROM PARASITIC NEMATODE *STRONGYLOIDES STERCORALIS*

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RIO protein kinases, a group of newly discovered atypical protein kinases (including three members: RIO1, RIO2 and RIO3) play important roles in cell-cycle progression, 20S pre-rRNA processing and development of many organisms. In spite of their functional significance, there is still a paucity of information on the function of RIO protein kinases in parasitic nematodes. In the present study, full-length cDNA and gDNA sequences of all three RIO protein-encoding genes, Ss-rio1, Ss-rio2 and Ss-rio3, were identified from the parasitic nematode, *Strongyloides stercoralis*. The full-length cDNA of Ss-rio1 comprises 1820 bp, including a 369 bp 5′ UTR, 17 bp 3′ UTR and a 1434 bp coding sequence (CDS). The full-length cDNA of Ss-rio2 is 1572 bp, encoding 524 amino acids. The full-length cDNA of Ss-rio3 has 1736 bp, including a 179 bp 5′ UTR, 36 bp 3′ UTR and a 1521 bp CDS. Two, one and no introns were found in gDNAs of Ss-rio1, Ss-rio2 and Ss-rio3, respectively. Moreover, the in silico analyses of three genes revealed that the promoters of Ss-rio1 with 3128 bp, Ss-rio2 with 1283 bp and Ss-rio3 with 600 bp, contain the conserved motifs, such as a CAAT box, a GATA box, a GC box, an E-box (CANNTG) and a TATA box. In addition, transcriptomic analysis revealed that Ss-rio1 and Ss-rio3 were transcribed at similar levels in most development stages and females of S. strongyloides whereas Ss-rio2 had higher transcriptional levels in free-living and parasitic females than in post free-living L1 and infective L3. Furthermore, the anatomical expression patterns of these three genes were also investigated in transgenic *S. stercoralis*. Ss-rio1 is expressed in some neurons, pharynx, hypodermis and tail phasmidial neurons while Ss-rio2 is expressed in body wall and intestine, and Ss-rio3 in some of the head neurons and hypodermis. The findings from the present study provide a first basis for elucidating molecular functions of RIO protein-encoding genes in the biological processes of parasitic nematodes.

## 493

### PREVALENCE OF *STRONGYLOIDES STERCORALIS* INFECTION IN A REMOTE INDIGENOUS COMMUNITY AS DETERMINED BY ELISA TESTING FROM DRIED BLOOD SPOT FOR ANTIBODIES TO THE RECOMBINANT ANTIGEN NIE

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Parasitologic diagnosis of infection with the intestinal nematode *Strongyloides stercoralis* is relatively insensitive and logistically challenging. Serologic assays based on detection of antibodies to crude larval antigen offer increased sensitivity, but specificity is hampered by cross-reactive antibodies and persistence after cure. Further, standardization of antigen for assays is problematic. The use of recombinant antigen can potentially overcome these problems, and the NIE antigen from *S.stercoralis* has been used widely with good diagnostic sensitivity and specificity. Detection of antibody eluted from dried blood spots has shown utility in large-scale seroepidemiologic studies, and is appealing in children where venipuncture or stool collection is problematic. We adapted an existing NIE-ELISA protocol for the testing of anti-strongyloides antibody response on dried blood spots collected as part of an ivermectin mass drug administration conducted in an Australian Indigenous community. The NIE ELISA was first validated using representative positive, negative, and equivocal time-matched serum samples previously tested using *S. ratti* antigen ELISA. Optimal assay and storage conditions were determined using positive control blood spots, and samples screened with the adapted NIE-DBS-ELISA. Blood spots were stable for several days at 37°C, or following longer-term storage at 4°C, -20°C or -80°C. The sensitivity of the NIE-DBS-ELISA was determined by ROC analysis to be 82%. Of the 219 blood spots tested, 18% were positive for *S. stercoralis*, a similar prevalence to that documented by standard *S. ratti* serology. In representative samples positive for *S. ratti-specific* antibodies, a significant decline in NIE optical density was observed at 6 and 12 months following ivermectin MDA ($p < 0.0001$). No time-associated differences were seen in negative or equivocal samples. This further confirms the high seroprevalence of *S. stercoralis* in remote Australian Indigenous communities, and suggests that collection of dried blood spots may be useful approach for field-friendly diagnosis of strongyloidiasis.

## 494

### *STRONGYLOIDES* HYPERINFECTION SYNDROME IN AN HTLV-1 CO-INFECTED HIV PATIENT

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An HIV-1 positive, ART naïve, 35- year old male presented to the HIV clinic in Lambaréné, Gabon, with complaints of hemoptysis, persistent after treatment with broad-spectrum antibiotics. Initially there were no constitutional symptoms, the X-thorax showed no particularities, and sputum microscopy was negative for acid fast bacilli. CD4 counts were remarkably high, with repeated values around 1500 cells/mm$^3$. The FBC

yielded a mild microcytic anaemia with a haemoglobin of 10.4 g/dL without further abnormalities. After local routine periodic treatment for intestinal parasites with albendazole 400 mg once (' deworming'), the clinical picture deteriorated and the patient developed fulminant diarrhea with weight loss over 10% of his initial body weight, vomiting and at a later stage paralytic ileus. The patient remained afebrile during the course of the illness. Rhabditiform larvae of *Strongyloides stercoralis* were identified in the sputum and numerous filariform larvae were found in the faeces. The patient was treated with ivermectin 200µg/kg body weight for 5 days, after which his clinical status improved dramatically. Subsequently, his CD4 count decreased to 100 cells/mm$^3$ and he was started on ART. Disseminated strongyloidiasis is a severe condition and is associated with corticosteroid use and HTLV-1 infection. HIV-infection does not predispose to the syndrome. In this case, infection with HTLV-1 might have been the reason for the upregulation of CD4 cells, triggered by the auto-infection process of *S. stercoralis*. This masked the severity of immunosuppression by HIV. Clinicians should consider the diagnosis of HTLV-1 in patients from endemic areas with disseminated Strongyloidiasis, both HIV-infected and -uninfected.

## 495

### HOW FREQUENTLY AND TO WHOM SHOULD MASS CHEMOTHERAPY BE ADMINISTERED TO CONTROL SOIL TRANSMITTED HELMINTHS?

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Large-scale treatment programmes for soil-transmitted helminths (STHs) are now being scaled up in endemic areas to reach children in need. There is a concomitant need to decide how best to utilise resources to achieve the biggest impact in terms of reducing transmission in the long term and for effective modelling to support this. We model four age-groups, <2 years, 2-4 years (pre-school-aged children, pre-SAC), 5-15 years (school-aged children, SAC) and adults. The different rates of acquiring infection and of depositing infective stages for the rest of the population to acquire are estimated by fitting the model to a number of age-stratified reinfection studies from countries in sub-Saharan Africa and SE Asia, and to cross-sectional data on intensity of infection by age from single points in time. We describe how the frequency and targeting of effective treatment strategies depend on the overall intensity of infection, the relative intensities of infection in different age groups and the species of helminth. Importantly, we show how the ideal treatment strategy depends on the aim and timescale of the treatment programme. In areas of low to medium transmission (classified on the basis of current WHO guidelines) long-term (>10 years), repeated biannual or annual treatment of >75% of SAC is likely to have large impacts on mean intensity of round worm infections in children and, under certain conditions, on the rest of the population. However, in areas of high transmission significant reductions across the population require the treatment of adults as well as pre-SAC and SAC children. We outline the design of optimum different strategies, extending from SAC to pre-SAC and then adults, under different epidemiologic conditions, including different combinations of Ascaris, Trichuris and hookworm. We also calculate the breakpoints in transmission in terms of the mean worm loads in different age groupings (pre-SAC, SAC and adults) below which treatment can cease. The results are then used to suggest some guidelines for community based control of STH by chemotherapy.

## 496

### HELMINTH INFECTIONS AND MICRONUTRIENTS IN SCHOOLCHILDREN: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Helminth infections and micronutrient deficiencies are both highly prevalent in developing countries. Neither condition typically causes overt disease but they do lead to indirect morbidity and impaired physical and cognitive development. We aimed to systematically review current evidence on the relationship of helminth infections with micronutrient status in schoolchildren worldwide. We included both observational studies and RCTs. We used random effects meta-analysis 1) to estimate cross-sectional associations between helminths and micronutrient status; 2) to estimate anthelminthic treatment effects on micronutrient status, and 3) to estimate effects of micronutrient supplementation on helminth (re)infection. Meta-analyses of observational studies showed a significant association between helminth infections and serum retinol (SMD (standardized mean difference) -0.30 [-0.48;-0.13]) but not serum ferritin (SMD 0.00 [-0.7;0.7]). Conversely, meta-analyses of anthelminthic RCT studies did show a positive effect on ferritin (SMD 0.14 [0.08;0.20]) but not on retinol (SMD 0.04 [-0.06;0.14]). We did not find enough studies to pool data on other micronutrients besides ferritin and retinol. When evaluating helminth (re)infection rates in micronutrient supplementation studies, only multi-micronutrient interventions showed a modest protective effect (OR 0.77 [0.61; 0.97]). In conclusion, we found significant associations between helminth infections and micronutrient status in schoolchildren. Our results showed distinct associations with either serum retinol or serum ferritin. More evidence is needed to further unravel the interrelationship between helminth infections and micronutrient status.

## 497

### TRANSMISSION-BLOCKING INTERVENTIONS ELIMINATE MALARIA FROM LABORATORY POPULATIONS

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Anti-malarial transmission-blocking interventions (TBIs) aim to reduce the prevalence of infection in endemic communities by targeting *Plasmodium* within the insect host. Whilst many studies have previously reported the successful reduction of infection in the mosquito vector, direct experimental evidence that there is an onward reduction in infection prevalence in the vertebrate host is lacking. We report the first experiments using a population, transmission-based study of *P. berghei* in *Anopheles stephensi* to assess the impact of a transmission-blocking drug upon both insect and host populations over multiple transmission cycles. We demonstrate that a selected TBI (atovaquone), which inhibits transmission from vertebrate to insect by only 32%, reduces the basic reproduction number of the parasite by 20%, and in our model system can eliminate *Plasmodium* from mosquito and mouse populations at low transmission intensities. These findings clearly demonstrate that use of TBIs alone can eliminate *Plasmodium* from a vertebrate population, and have significant implications for the future design and implementation of TBIs within the field.

## 498

### TARGETING SEXUAL STAGE MALARIA PARASITES WITH TRANSMISSION-BLOCKING COMPOUNDS IN THE MOSQUITO MIDGUT

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To achieve malaria elimination/eradication, the consensus opinion is that new approaches to drug design are desperately needed. We have undertaken two approaches towards the development of novel malaria transmission-blocking drugs based on the strategy of inhibiting *Plasmodium* development in the mosquito gut either (i) by interfering with obligate cellular interactions between the parasite and the mosquito-midgut epithelium using small molecules that mimic midgut-surface ligands or (ii) by targeting sexual stage parasites with novel compounds that have antimalarial properties. For the first approach, we successfully designed a transmission-blocking small molecule (VS1) that mimics the structure of glycosaminoglycans (GAG), which serve as putative ligands for parasite attachment prior to midgut-cell invasion. For the second approach, we conjugated usinic acid, a dibenzo-furandione acylphloroglucinol derived from lichens with antiparasitic activity, to a number of other compounds including enamines and hydrazones with the goal of improving usinic acid solubility, while enhancing its antimalarial activity and reducing toxicity. Using feeding assays in which mosquitoes were fed with infectious blood, we tested the effect of VS1 and the usinic acid derivatives on *Plasmodium* development in the mosquito and found that the GAG mimetic and multiple usinic acid conjugates dramatically reduced infection intensity in the mosquito. We investigated the molecular mechanism of VS1 using a variety of approaches and found that VS1 binds to the circumsporozoite- and TRAP-related protein (CTRP), a protein expressed by ookinetes essential for midgut invasion. The modes of action of the usinic acid derivatives are currently under investigation, but usinic acid alone has been shown to be a membrane disruptor and to behave as a bifunctional antioxidant-pro-oxidant, depending on its concentration. Both of our approaches have yielded compounds that profoundly inhibit a key step of parasite development, thereby abrogating downstream events necessary for mosquito-to-human transmission.

## 499

### OWNERSHIP AND USE OF INSECTICIDE TREATED NETS AFTER MASS DISTRIBUTIONS TARGETING COVERAGE OF ALL SLEEPING SPACES

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Senegal has made great progress in malaria control through the scale up of malaria prevention and case management interventions and achieved a decrease in all cause child mortality of 40% from 2005 to 2010. In 2010, the Senegal National Malaria Control Program and partners began the implementation of a strategy for universal coverage of long lasting insecticide treated nets (LLINs) with a rolling campaign that covered all 14 regions over three years. Distribution was based on a sleeping space census conducted just before the campaign in each region, with the goal of ensuring one net per sleeping space. Pre-existing nets in good condition counted against the total to be distributed. From May 2010 through March 2013, 6,536,623 LLINs were distributed to 1,480,216 households. A cross sectional cluster sample household survey of 1561 households was conducted in July 2011 in the first six regions (covered from May 2010 through March 2011) to measure campaign coverage, net ownership, and use. The survey found 89% of households received at least one LLIN, with a mean of 4.0 LLINs per household. At the time of the survey, household ownership of at least one LLIN was 94%, and 42% of households owned at least one LLIN per sleeping space. The night before the survey, there were 0.79 LLINs per occupied sleeping space, 82.5% of campaign LLINs were hanging, and 71% of sleeping spaces were protected by an LLIN. Use of LLINs by children under 5 years and pregnant women was 72% and 74%, respectively. Among the general population, 69% in all households and 90% in households with at least one LLIN per sleeping space slept under an LLIN the previous night, demonstrating universal coverage by use when universal coverage by ownership was achieved. While short of the target of 80% use among the general population, Senegal has achieved high LLIN ownership and use. In 2013, a multichannel routine distribution system will be implemented in all regions and the rolling universal coverage campaign will return to the same regions covered in 2010 to maintain high net ownership and use.

## 500

### ASSOCIATION BETWEEN HOUSEHOLD INSECTICIDE-TREATED NET OWNERSHIP AND ALL-CAUSE CHILD MORTALITY IN MALAWI, 2007-2010

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Malawi has made major progress in scaling-up insecticide-treated bed net (ITNs) coverage over the past six years. Demonstrating the protective effectiveness of household ITN ownership for preventing all-cause child mortality (ACCM) under routine program conditions is an important step in the causal pathway towards assessing population-level impact. We used data from the 2010 Demographic and Health Survey (DHS) to assess whether household ownership of an ITN protected against ACCM in Malawi from 2007-2010. We calculated ITN ownership and ACCM retrospectively over this period from the DHS household net rosters, which includes when each net was obtained and/or retreated, and mother's birth histories. Several forms of bias are inherent to this approach. We attempted to mitigate bias due to confounding through exact matching of individuals with and without an ITN based upon residence (urban/rural), mother's education, cluster level vaccination coverage, malaria transmission level, and cluster distance to the nearest health facility. We then evaluated the association between ITN ownership and ACCM in a shared-frailty Cox proportional hazards model while additionally controlling for household wealth, child's age, mother's age, calendar year, parity, cluster level diarrhea prevalence, season (high/low transmission), and monthly rainfall and temperature. The resultant retrospective cohort included 29,492 children <5 who provided 652,775 child-months of observation and among whom there were 821 deaths over the observation period. After controlling for confounders, children in households with an ITN were significantly less likely to die compared to those without one (Hazard ratio (HR): 0.75, 95% confidence interval [CI]: 0.62-0.90). Additionally, ITNs reported as less than 1.5 years old provided greater protection than older nets (HR <1.5 yrs: 0.73, 95% CI: 0.60-0.88, >1.5 yrs: 1.18, 95%CI: 0.70-1.97). These results demonstrate the impact of household ITN ownership on ACCM in this setting and suggest that ITN coverage in Malawi may have contributed significantly to decreased child mortality.