

salivary glands was greatly reduced by ron2 silencing, despite sporogony, sporozoite release into hemocoel and their motility were normal. These results showed that RON2 is required for salivary gland invasion. This is the first genetical approach to show that RON2 has an important role in target cell invasion.

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### IDENTIFICATION AND CHARACTERIZATION OF A *PLASMODIUM FALCIPARUM* ORTHOLOGUE OF THE YEAST UBIQUINONE-BINDING PROTEIN, COQ10P

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Coenzyme Q (CoQ, ubiquinone) is a central electron carrier in mitochondrial respiration. CoQ is synthesized through multiple steps involving a number of different proteins. The prevailing view that the CoQ used in respiration exists as a free pool that diffuses throughout the mitochondrial inner membrane bilayer has recently been challenged. In the yeast *Saccharomyces cerevisiae*, deletion of the gene encoding Coq10p results in respiration deficiency without altering total size of the available CoQ pool, suggesting that the Coq10p is critical for the delivery of CoQ to the site(s) of respiration. The precise mechanism by which this is achieved remains unknown at present. Because mitochondrial respiration is a validated target for antimalarial drugs such as atovaquone, we are interested in examining its regulation in malaria parasites. We have identified an orthologue of Coq10p, PfCoq10, in *P. falciparum*, the most virulent species of malaria parasite, and demonstrated that a GFP-tagged version of PfCoq10 localized to the parasite mitochondrion. Expression of PfCoq10 in the *S. cerevisiae* coq10 deletion strain restored the capability of the yeast to grow on respiratory substrates, suggesting a remarkable functional conservation of this protein over a vast evolutionary distance, and despite a relatively low level of amino acid sequence identity. We are currently assessing effects of PfCoq10 overexpression on the atovaquone sensitivity of *P. falciparum*. We are also examining the possibility of altered response to atovaquone in yeast mitochondria expressing the parasite Coq10. These studies may provide insights into respiration regulation in general, as well as in malaria parasites.

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### ROLE OF PFRAD54 AND REPLICATION PROTEIN A IN RAD51-MEDIATED DNA STRAND EXCHANGE AND REPAIR OF DNA DAMAGE INDUCED BY MMS IN *PLASMODIUM FALCIPARUM*

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Exploiting the recombination machinery and its molecular characterization in the malaria parasite would provide mechanistic understanding of recombinational rearrangements leading to immune evasion via antigenic switching, a major impediment in developing an effective vaccine against these protozoan parasites. Bacterial RecA protein and its eukaryotic homologue Rad51 play a central role in homologous DNA strand exchange reaction during recombination and DNA repair. Previously, our lab has shown that PfRad51, the *Plasmodium falciparum* homologue of Rad51, exhibited ATPase activity and promoted DNA strand exchange *in vitro*, as reported previously. Here, we evaluated the catalytic functions of PfRad51 in the presence of putative interacting partners, especially *P. falciparum* homologues of Rad54 and Replication protein-A (RPA). PfRad54 accelerated PfRad51 mediated pairing between ssDNA and its homologous linear dsDNA in the presence of 0.5mM CaCl<sub>2</sub>. We also present evidence that recombinant PFRPA1L protein serves the function of bacterial homologue SSB in initiating homologous pairing and strand exchange activity but its function was negatively regulated in a dose-dependent manner by PFRPA1S, another RPA homologue in *P. falciparum*. We also present *in vivo* evidence through comet assays for methyl methanesulfonate (MMS)- induced DNA damage in malaria parasites

and accompanying upregulation of PfRad51, PfRad54, PFRPA1L and PFRPA1S at the level of transcript and protein. This study provides new insights into the role of putative Rad51-interacting proteins involved in homologous recombination and emphasizes physiological role of DNA damage repair during the growth of parasites. We are now characterizing the recombination macromolecular complex which is likely to be important in DNA damage and repair and validating molecular interactions between PfRad51 and its putative interacting partners. Besides understanding molecular machinery involved in DNA repair and recombination, we wish to extend our studies to understand the biochemical and genetic basis of gene rearrangements at the var gene locus associated with phenomenon like antigenic variation.

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### A SINGLE NUCLEOTIDE POLYMORPHISM IN THE PROMOTER OF STROMAL CELL-DERIVED FACTOR (SDF)-1A (C-1002T) IS ASSOCIATED WITH PROTECTION AGAINST *PLASMODIUM FALCIPARUM* INFECTION IN KENYAN CHILDREN

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Stromal cell-derived factor (SDF)-1 $\alpha$  (CXCL12) is a pleiotropic chemokine with diverse functions including induction of anti-pathogen immunity and inhibition of erythropoiesis. In murine malaria, increased expression of SDF-1 $\alpha$  promotes control of parasitemia. Although several studies indicate that *SDF1A* genetic variation regulates outcomes in the context of HIV-1 infection, hematopoiesis, and cancer, the role of genetic variability in *SDF1A* in *Plasmodium falciparum* infections has not been explored. The effect of *SDF1A* (C-1002T, rs2839686) variation was therefore, investigated in Kenyan children (2.0-38.0mos., n=873) residing in a holoendemic *P. falciparum* transmission region of western Kenya. Children were stratified into aparasitemic (n=212) and parasitemic (n=661) groups with parasitemic children being further categorized into SMA (hemoglobin, Hb<5.0g/dL; n=236) vs. non-SMA (Hb $\ge$ 5.0g/dL; n=425), high-density parasitemia (HDP;  $\ge$ 10,000 parasites/ $\mu$ L; n=477) vs. low-density parasitemia (LDP; <10,000 parasites/ $\mu$ L; n=184), and reticulocyte production index (RPI<2.0) vs. (RPI $\ge$ 2.0). Multivariate logistic regression modeling controlling for age, gender, bacteremia, glucose-6-phosphate dehydrogenase, alpha-thalassemia, and sickle cell and HIV-1 status did not show any significant associations between carriage of C-1002T genotypes and SMA, RPI<2.0, and HDP. However, carriage of the CC genotype was associated with protection against the acquisition of *P. falciparum* infection compared to the TT genotype (Odds ratio, OR, 0.311; 95% CI, 0.115-0.842; P=0.022). These results demonstrate that although variation at 1002 in the *SDF1A* promoter appears to protect against acquisition of *P. falciparum* infection, this variant may not affect malaria disease outcomes once an individual becomes infected.

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### GENOMIC DIVERSITY AND EVOLUTIONARY HISTORY OF *PLASMODIUM VIVAX*

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Most studies of genetic diversity in *Plasmodium vivax* have focused on microsatellites or selected loci and do not provide a genome-wide perspective. We have sequenced the genomes of ten *P. vivax* field isolates

collected across the world, and three monkey-adapted strains. We sequenced each sample on one lane of an Illumina HiSeq 2000 to obtain 70–400X coverage and have >93% of the reference genome covered by >20 reads. We conservatively identified >85,000 SNPs across the genome as well as several sequence rearrangements. The genetic diversity is significantly higher in intergenic regions than in coding regions (6.84 vs. 3.24 SNPs/kb) with intronic sequences harboring an intermediate level (4.63 SNPs/kb). We also observed 1.5-fold more non-synonymous than synonymous SNPs while, based on the genome composition, we would expect 4-fold more nsSNPs. These observations suggest that evolution of most *P. vivax* protein-coding sequences is driven by purifying selection. To identify genes evolving under positive selection, we compared the rates of non-synonymous (PN) and synonymous substitutions (PS) for all protein-coding sequences. Two red blood cell binding protein genes, the Duffy binding protein and the reticulocyte binding protein 2 like, were among the ten genes with the highest PN/PS ratio (2.14 and 1.89 respectively), suggesting that they are evolving under strong positive selection. The reticulocyte binding protein genes 1 and 2 also displayed PN/PS ratios consistent with positive selection. The PN/PS ratio for other red blood cell binding proteins, such as the apical merozoite antigen 1 or the rhoptry neck protein 1 and 2 genes, was more consistent with negative selection. Our genome-wide analyses of selective constraints in *P. vivax* suggest that natural selection is actively changing the amino acid sequences of proteins involved in early stages of erythrocyte invasion (merozoite attachment to red blood cells and reorientation), while proteins involved in latter stages (junction formation and invasion) are more conserved.

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### GENOME-WIDE ANALYSIS OF NATURAL SELECTION IN *PLASMODIUM FALCIPARUM* POPULATIONS IN WEST AFRICA

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When *Plasmodium falciparum* competes with other genotypes in same host or when under drug and host immunity pressure there is selection on the genome which leaves marks that can be identified by population genetic methods. Selective pressure will vary in different geographical locations and the varying marks detected in this case will correspond to genes involved in local adaptation. To explore this, we first analysed population structure by genotyping ten polymorphic *P. falciparum* microsatellite loci in 268 infections from eight locations in four West African countries (Republic of Guinea, Guinea Bissau, The Gambia and Senegal), spanning a highly endemic forested region in the south to a low endemic Sahelian region in the north. This showed that each location had similar levels of genotypic diversity, although there were many more mixed parasite genotype infections in the south. Genetic differentiation between populations was low and an overall test for isolation by distance was not significant. Given the high levels of recombination and minimal reproductive isolation of parasite populations in West Africa, differential signatures of selection in particular populations should be detectable against a background of neutral genomic variation that is more spatially homogeneous. To address this, we undertook a population genomic analysis of parasites at a highly endemic site in the Republic of Guinea, with whole genome sequencing using Illumina 76 base paired-end reads mapped to the reference genome of *P. falciparum*. Genome-wide analysis of SNPs in 100 *P. falciparum* clinical isolates from this population was used to identify genes under natural selection, and we compare the findings with those of a lower endemic site in The Gambia.

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### TRACKING CHANGING POPULATION DYNAMICS OF *PLASMODIUM FALCIPARUM* INFECTIONS REVEALS EVIDENCE OF CLONAL INFECTIONS FOLLOWING INTRODUCTION OF TRANSMISSION-REDUCING INTERVENTIONS IN THIÉS, SENEGAL

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As global health efforts to control and eliminate malaria gain momentum, genetic tools can aid in monitoring malaria transmission dynamics, detecting emergence of drug resistant parasites, and identifying sources of new malaria infections. Using genotyping tools, we identified a large number of clonal infections among patients with mild uncomplicated malaria seeking treatment at a clinic in Thiés, Senegal. While it is not unexpected to find limited instances of the same parasite within households or related individuals, the occurrence of identical parasites has been increasing since 2006 (P-value = 0.006), with more than 25% of the parasites collected from geographically and temporally distinct patients sharing an identical molecular barcode in 2008 and 2011. We validated the sensitivity of the molecular barcode to detect parasites that are nearly genetically identical by sequencing and hybridization to Affymetrix SNP genotyping arrays. These clonal findings are coincident with increased deployment of malaria control interventions and decreased malaria deaths in Senegal from 2006 to 2011. In addition, parasite types identified in one transmission season were present in subsequent seasons, providing evidence that specific parasite lineages persist across years. Further, the appearance and rise of clonal parasites corresponded with a substantial decrease in the effective parasite population size calculated by several methods from a high of over 106 parasites in 2006 to less than 40 by 2011. These data provide the first evidence of a strong temporal correlation between the appearance of clonal parasite types and increased malaria control interventions including distribution of insecticide treated nets; use of rapid diagnostic tests for malaria detection; and deployment of artemisinin combination therapy approaches. Our results demonstrate that population genetic based tools can be used as a diagnostic tool to detect changes in malaria intensity and evaluate the effectiveness of local control and elimination strategies to best inform a global malaria eradication campaign.

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### WOLBACHIA-BASED INTERVENTIONS FOR MALARIA VECTOR CONTROL

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Malaria, transmitted by *Anopheles* mosquitoes, remains a major global public health problem. Novel vector control interventions are urgently needed to address vector species that are not effectively targeted by current tools. *Wolbachia* is a maternally transmitted symbiotic bacterium that can not only spread within mosquito populations through its unique ability to manipulate mosquito reproduction but can also induce resistance to human pathogens in mosquito vectors. We have now successfully established a stable *Wolbachia* infection in a major Asian malaria vector, *Anopheles stephensi*. This vector-resident *Wolbachia* has a 100% maternal transmission rate and induces nearly complete cytoplasmic incompatibility. Laboratory population replacement experiment shows *Wolbachia* could reach 100% infection frequency within seven generations after release.

These results open the possibility to use *Wolbachia* as a novel intervention tool for malaria control, as successfully demonstrated in *Aedes* mosquitoes.

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### WOLBACHIA USES HOST MICRORNAS TO FACILITATE COLONIZATION OF THE DENGUE VECTOR AEADES AEGYPTI

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*Wolbachia* are maternally inherited, gram-negative endosymbiotic bacteria, which are commonly found in invertebrates. It is estimated that more than 65% of all insect species are infected with *Wolbachia*. They are best known for manipulating the host reproductive systems through a variety of strategies such as cytoplasmic incompatibility, male-killing, feminization and parthenogenesis. The *Wolbachia* strain wMelPop has been shown to be able to modulate lifespan of host insects and interfere with development of human pathogens in mosquito vectors. However, very little is known about the molecular basis of the interactions. Understanding the mechanisms involved in the interactions may facilitate devising novel approaches to inhibit/limit transmission of mosquito-borne pathogens. Using microarrays and deep sequencing, we have shown that the endosymbiont manipulates the host mosquito's (*Aedes aegypti*) microRNA (miRNA) profile to facilitate its replication/maintenance in the host cells. In addition, alterations in miRNA and cellular proteins involved in miRNA trafficking are observed in *Wolbachia*-infected mosquitoes. Results pertaining identification and functional characterization of a number of miRNAs manipulated by *Wolbachia* that play significant roles in host colonization and biology will be discussed.

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### IVERMECTIN INHIBITS AND DELAYS THE DEVELOPMENT OF PLASMODIUM FALCIPARUM IN ANOPHELES GAMBIAE

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Multiple ivermectin mass drug administration (MDA) to humans in southeastern Senegal reduced both the survivorship of *Anopheles gambiae* s.s. and the proportion of *Plasmodium falciparum*-infectious *An. gambiae*. Ivermectin has been shown to impact several factors of vectorial capacity including: the time between blood meals, the daily probability of mosquito survivorship, and by extension the vector-to-host ratio. Here we investigated whether ivermectin alters two other factors of vectorial capacity, the proportion of *An. gambiae* that become infective and the time for parasite development. Indeed, ivermectin inhibits and delays the development of *P. falciparum* (NF 54) in *An. gambiae* (G3). Ivermectin was fed to *An. gambiae* at the lethal concentration that kills 25 percent of mosquitoes (LC<sub>25</sub>). Mosquitoes were dissected on days post infection (DPI) 7, 12 and 14. When ivermectin and *P. falciparum* were co-ingested at the same time the proportion of vectors with oocysts (LC<sub>25</sub> DPI 7, P=0.0013) and sporozoites (LC<sub>25</sub> DPI 12, P=0.0286, LC<sub>25</sub> DPI 14, P=0.00133) was reduced. Development of *P. falciparum* in the vector was delayed as evidenced by analysis of rates of change in the proportion of control mosquitoes between DPI 12 = 0.5648 and DPI 14 = 0.7720 (P=0.0651) and treatment mosquitoes between DPI 12 = 0.1933 and DPI 14 = 0.4342 (P=0.0486). Development in the vector was inhibited when ivermectin was ingested six DPI (LC<sub>25</sub> DPI 14, P=0.0317), but not three DPI (LC<sub>25</sub> DPI 14, P=0.7193). This suggests that ivermectin may inhibit development of late stage but not early stage oocysts. This work demonstrates that ivermectin inhibits oocyst development and is sporontocidal, although the mechanism of action has not been determined yet. Ivermectin MDA is the only malaria transmission control tool currently available that can impact all factors of vectorial capacity. Future work will utilize field isolates of *P. vivax* and *P. falciparum* to determine the impact of ivermectin on *Plasmodium* development in Thai malaria vectors.

### THE EXPRESSION OF OLFACTION GENES IN ANOPHELES GAMBIAE IN RELATION TO HUMAN HOST PREFERENCE

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Females of *Anopheles gambiae*, the main vector of human malaria in Sub-Saharan Africa, have a strong attraction to odors of humans, their primary host. Several gene families involved in the olfaction system; the olfactory receptors (ORs), Ionotropic receptors (IRs), and odorant binding proteins (OBPs), interact directly with odorants. As such they are prime candidates for contributing to the strong human host preference of *An. gambiae*. To understand the molecular basis of anthropophily in *An. gambiae*, we are examining the differential expression of olfaction genes in *An. gambiae* and *An. quadriannulatus*, a zoophilic species within the *An. gambiae* species complex. Our expectation is that olfaction genes important in human host preference will be expressed at higher relative levels in *An. gambiae* compared to *An. quadriannulatus*. Using Illumina high-throughput sequencing, we have generated transcriptome data from the heads (including antennae and maxillary palps) of backcrosses between the two species. Backcross female host preference was assessed using an olfactometer. Two RNA-seq libraries consist of backcross females who prefer human hosts, and two RNA-seq libraries consist of backcross females who prefer cow hosts. We identified 31 olfaction genes that are expressed more than two-fold in the human vs the cow preferring pool: 20 ORs, 10 OBPs, and 1 IR. If these up-regulated olfaction genes are involved human host preference, we also expect them to be expressed at higher levels in the antennae/palps of *An. gambiae* females compared to males or *An. quadriannulatus*. We have therefore analyzed the transcriptomes of both the antennae and maxillary palps from the females and males from both species. This study allows us to identify candidate genes responsible for the adaptation of *An. gambiae* to human hosts and that may provide promising targets for designing repellents/attractants or transgenic approaches for controlling *An. gambiae* populations.

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### EXPRESSION OF THE OLFACTION GENE REPERTOIRE IN AEADES AEGYPTI FOLLOWING BLOOD FEEDING

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Mosquito host preference is a complex trait that is closely tied to disease transmission and presumably involves the precise regulation of numerous olfaction related genes. The yellow fever mosquito *Aedes aegypti* displays considerable behavioral and physiological changes after acquiring a blood meal and females suppress host seeking behavior following a blood meal. Numerous genes belonging to three families; the olfactory receptors (ORs), the ionotropic receptors (IRs), odorant binding proteins (OBPs) are potentially involved in host seeking behavior and are expressed in the antennae. We expect unfed female *Ae. aegypti* to express genes needed to locate human hosts, whereas those that are blood fed do not, or do so at a reduced level. RNA-seq was used to quantify changes in the expression of protein coding genes in the main olfaction organs of *Ae. aegypti*, the antennae. Four days old females were blood fed and antennae were collected prior to and 3, 24, 48 and 72h after feeding. Differential expression analyses were performed using the unfed females as a reference. The total number of genes differentially expressed were 63, 111, 107, and 94 for 3, 24, 48 and 72h, respectively. Three gene families were analyzed in detail: Olfaction Receptors (ORs), Ionotropic Receptors (IRs), and Odorant Binding Proteins (OBPs). Most (62.5%) of the OBPs are highly expressed (FPKM>1000), whereas most IRs (65%) are not expressed in the antenna of females. ORs tend to be expressed at intermediate levels; 55.1% of annotated ORs with FPKM from 10 to 100. Most of the ORs are down-regulated after feeding, but typically the

changes in expression are small. The expression profile of OBPs and IRs does not change considerably after feeding compared to ORs. Genes of particular interest are OR46, OR99, and one IR (AAEL005039), that are expressed in unfed females only. Genes differentially expressed or those only expressed before or after feeding, are candidate host seeking genes.

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### IGG RESPONSES TO ANOPHELES GAMBIAE SALIVARY ANTIGEN GSG6 DETECT VARIATION IN EXPOSURE TO MALARIA VECTORS AND PREDICT DISEASE RISK

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Assessment of exposure to malaria vectors is important to our understanding of spatial and temporal variations in disease transmission and facilitates the targeting and evaluation of control efforts. Recently, an immunogenic *Anopheles gambiae* salivary protein (gSG6) was identified and proposed as the basis of an immuno-assay determining exposure to Afrotropical malaria vectors. In the present study, IgG responses to gSG6 and 6 malaria antigens (CSP, AMA-1, MSP-1, MSP-3, GLURP R1, and GLURP R2) were compared to anopheles exposure and malaria incidence in a cohort of children from Korogwe district, Tanzania; an area of moderate and heterogeneous malaria transmission. Anti-gSG6 responses above the threshold for sero-positivity were detected in 15% (96/636) of the children, and were positively associated with geographical variations in *Anopheles* exposure (OR 1.25, CI 1.01-1.54, p=0.04). Additionally, IgG responses to gSG6 in individual children showed a strong positive association with household level mosquito exposure. IgG levels for all antigens except AMA-1 were associated with the frequency of malaria episodes following sampling. gSG6 seropositivity was a strong positive indicator of subsequent malaria incidence (test for trend p=0.004), comparable to malaria antigens MSP-1 and GLURP R2. Our results show that the gSG6 assay is sensitive to micro-epidemiological variations in exposure to *Anopheles* mosquitoes, and provide a correlate of malaria risk that is unrelated to immune protection. While the technique requires further evaluation in a range of malaria endemic settings, our findings suggest that the gSG6 assay may have a role in the evaluation and planning of targeted and preventative anti-malaria interventions.

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### TAXIS BOXES DETECT LONG-DISTANCE DIRECTIONAL MOVEMENT OF MOSQUITOES TO OLFACATORY CUES

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Malaria control methods targeting indoor-biting mosquitoes are increasingly compromised by insecticide resistance and have limited impact on vectors that feed and rest outdoors. Exploiting mosquito olfactory behaviour to reduce blood-feeding outdoors might be an evolutionarily sustainable approach to complement existing control strategies. Methodologies that can objectively quantify long-range responses to odour under realistic field conditions and allow high-throughput screening of many compounds are required for development of effective odour-based control strategies. The olfactory responses of *A. arabiensis* females (N = 1920/treatment) in an outdoor field setting to four treatments at

four distances up to 100 metres were measured using three-chambered taxis boxes that allow mosquito responses to natural or experimentally-introduced odour cues from the surrounding environment to be quantified under field conditions. Taxis box assays reliably detected directional movement of mosquitoes with more being attracted to natural and blended synthetic human odours than negative controls. However, CO<sub>2</sub> alone elicited no response in *A. arabiensis*. Attraction to stimuli decreased with increasing distance away from the point of stimulation. The range of attraction of mosquitoes to synthetic and human odour extended to 77 and 103 metres respectively. We have developed a reproducible and simple system to allow for the comparison of compounds that are active over medium- to long-ranges in a full field environments. The long natural range of attraction of anopheline mosquitoes to potential blood sources has substantial implications for the design of malaria control strategies, and adds to the understanding of long-distance olfactory behaviour in mosquitoes. While such investigations of malaria vector ecology have enormous potential in their own right, this experimental strategy could also be applied to other motile arthropods of medical, veterinary and agricultural significance.

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### A PROSPECTIVE STUDY ON POTENTIAL CAUSES OF DIARRHEA IN BANGLADESHI CHILDREN IN THE FIRST YEAR OF LIFE USING A PCR-LUMINEX BASED DETECTION OF 28 MOST COMMON ENTEROPATHOGENS

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We developed a PCR-Luminex based assay to detect 28 most common organisms associated with diarrhea. The enteropathogens included were *Cryptosporidium* spp., *Entamoeba histolytica*, *Giardia*, Microsporidia, *Cystoisospora*, *Cyclospora*, *Ascaris*, Hookworms, *Strongyloides*, *Trichuris*, *EPEC*, *EHEC*, *EIEC*, *EPEC*, *EPEC*, *Salmonella* (pan), *Campylobacter* (*C. coli* and *C. jejuni*), *Shigella* (pan), *Yersinia* (pan), and *Vibrio* (*V. cholera* and *V. parahaemolyticus*), *Astrovirus*, *Norovirus* G1 and G2, *Rotavirus*, *Sapovirus*, *Adenovirus*, and an internal control. This panel was used in a prospective study to determine etiology of diarrhea in Bangladeshi children in the first year of life. Specific primers and probes were designed for the organisms using targets which are conserved. DNA or RNA purified from stool was amplified using biotinylated primers, followed by hybridization to amine-modified probes covalently linked to carboxylated spectrally-distinct microspheres, followed by addition of streptavidin PE to detect specifically-bound amplicon. Luminex results are reported as corrected mean fluorescence intensity (cMFI) normalized to background, where cMFI > 2.5 was utilized as a "present" call with the exception of *Cryptosporidium*, *Strongyloides*, all *E. coli*, and all viruses where cMFI values were 7.3, 9.0, 10.0, and 2.0, respectively. Performance of the assays yielded 95% to 100% sensitivity and specificity versus the assays performed via real-time PCR or other conventional PCR. We then applied the tests in a prospective study of 147 children from Mirpur, Bangladesh followed monthly for the first year of life. 83% of the children had at least 1 episode of diarrhea in the first year of life and 33% had 4 or more. The number of pathogens that were detected increased as the number of diarrheal episodes increased. *Rotavirus*, *Giardia*, *Campylobacter*, and *Trichuris* were the leading pathogens detected during the first diarrheal episodes, while *Adenovirus*, *Campylobacter*, *Shigella*, *EPEC*, *EPEC*, and *Giardia* emerged as common in the later episodes. This Luminex based assay for the major enteropathogens offers sensitive and specific detection similar to real time PCR. When applied to field studies in endemic areas, a singular etiology of diarrhea is difficult to determine due to the frequency of mixed infections, and multiple pathogens may be the norm. Pathogens appear to accumulate in children that develop recurrent diarrhea.

### CHARACTERISTICS AND RISK FACTORS OF MODERATE-TO-SEVERE DIARRHEA OF PROLONGED OR PERSISTENT DURATION AMONG CHILDREN LESS THAN FIVE YEARS OLD IN RURAL WESTERN KENYA ENROLLED IN THE GLOBAL ENTERICS MULTICENTER STUDY (GEMS), 2008-2011

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We examined characteristics and risk factors for prolonged or persistent diarrhea among Kenyan children <5 years old participating in GEMS. Children presenting at a clinic with acute moderate-to-severe diarrhea were enrolled. Data on diarrhea duration were reported by the child's caretaker at enrollment for the previous 7 days and were recorded for 14 days after enrollment. Children were measured for weight and height at enrollment and at a 60-day follow-up, and stool specimens were tested at enrollment. We defined an episode of acute diarrhea (AD) as  $\leq 6$  days, prolonged acute diarrhea (ProAD) as 7-13 days, and persistent diarrhea (PD) as 14-20 days; the end of an episode was defined by 2 consecutive diarrhea-free days. We conducted logistic and ordinal logistic regression analysis; it was limited to cases with complete data on diarrhea duration and a single episode of diarrhea. From January 31, 2008 to February 6, 2011, 841 children met these criteria. Of these, 494 (59%) had AD, 285 (34%) had ProAD, and 62 (7%) had PD. Infants (OR: 2.0, CI: 1.4-2.8) and toddlers (OR: 1.9, CI: 1.3-2.8) were more likely to have diarrhea of longer duration as compared to 3-5 year olds (*Referent*). Longer durations of diarrhea were more likely to have occurred among children who were offered less than usual to drink while ill (OR: 1.3, CI: 1.0-1.8) and those who were moderately wasted at follow-up (OR: 1.9, CI: 1.1-3.1). Children who were stunted at enrollment (OR: 2.2, CI: 1.3-3.9) were more likely to have PD. Among children with a single pathogen identified (n=331) in their stool specimen, children with *Cryptosporidium* were more likely to have PD (OR: 4.5, CI: 1.3-13.6). Conversely, *Giardia* infection (OR: 0.4, CI: 0.2-0.8) was associated with diarrhea of shorter duration. Diarrhea duration appears to be multifactorial, influenced by a child's age, nutritional status, feeding practices while ill, and the infectious agent. Improved nutrition and appropriate hydration during illness may reduce the consequences and length of a diarrheal episode.

### SEQUELAE OF MODERATE-TO-SEVERE DIARRHEA AMONG YOUNG CHILDREN IN WESTERN KENYA, 2008-2011

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Diarrheal disease remains a major cause of morbidity and mortality among children in developing countries. Diarrheal episodes may predispose children to other illnesses like respiratory infections, but the risk and nature of these complications are poorly quantified. We assessed

the risk of various sequelae following an episode of moderate-to-severe diarrhea (MSD) in Kenyan children using data from the Global Enterics Multi-Center Study (GEMS), a 3-year prospective case-control study of children <5 years old presenting at a health facility with MSD ( $\geq 3$  loose stools in the last 24 hours, within 7 days of illness onset, with  $\geq 1$  of the following: sunken eyes, skin tenting, dysentery, IV rehydration, or hospitalization). Control children were matched on age, sex, and village and were diarrhea-free during the 7 days before enrollment. We assessed children at home 60 days after enrollment to obtain information on intercurrent illnesses and complications. We calculated matched odds ratios (mOR) adjusted for age using conditional logistic regression. Between January 2008 and January 2011, 1,473 case and 1,883 control children were enrolled. Within 60 days of presentation with diarrhea, case-patients were significantly more likely than controls to report experiencing an additional diarrheal episode (mOR 3.4, 95% CI 2.9 - 4.1,  $p < 0.0001$ ), dysentery (mOR 2.6, 95% CI 1.5 - 4.9,  $p = 0.0006$ ), or a seizure (mOR 1.8, 95% CI 1.1 - 3.1,  $p = 0.03$ ), and to report cough with difficulty breathing or a clinical diagnosis of pneumonia (mOR 1.3, CI 1.1 - 1.5,  $p = 0.007$ ) or to report being diagnosed with malaria (mOR 1.2, 95% CI 1.0 - 1.4,  $p = 0.01$ ). Additionally, case-patients were more likely to have a weight-for-height z-score  $< -2$  (mOR 2.0, 95% CI 1.6 - 2.6,  $p < 0.0001$ ) or height-for-age z-score  $< -2$  (mOR 1.2, 95% CI 1.1 - 1.4,  $p = 0.003$ ) at follow-up. Increasing diarrhea prevention efforts and careful follow-up of children with MSD may have broad and substantial impact on child morbidity and mortality in developing countries.

### CRYPTOSPORIDIUM INFECTION IN CHILDREN LESS THAN FIVE YEARS OLD WITH MODERATE-TO-SEVERE DIARRHEA IN RURAL WESTERN KENYA, 2008-2011

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Recent data from the Global Enterics Multicenter Study (GEMS), a 3-year case-control study of children <5 years with moderate-to-severe diarrhea (MSD) in seven countries, suggest that *Cryptosporidium* is a major contributor to infant diarrheal illness in Africa and Asia. GEMS cases were children <5 years who presented at a clinic with MSD ( $\geq 3$  loose stools in the last 24 hours, within 7 days of illness onset, with  $\geq 1$  of the following: sunken eyes, skin tenting, dysentery, IV rehydration, or hospitalization). Controls were age-, gender-, and village-matched, and diarrhea-free during the 7 days before enrollment. Stool samples were tested at enrollment; data on water, sanitation and hygiene were collected, and each child's health status was recorded at a 60-day follow-up interview. Presence of diarrhea was recorded by caretakers for 14 days following enrollment. From January 31, 2008 to January 29, 2011 in the rural GEMS Kenya site, *Cryptosporidium* was identified in 160 (10.8%) case children and 88 (4.7%) controls. Prevalence of *Cryptosporidium* was 10.6% among cases <6 months old, 16.2% among cases 6-11 months old, and 8.1% among cases 12-59 months old. Among cases with a single pathogen identified, those with *Cryptosporidium* had a significantly longer median duration of diarrhea than those with another diarrheal pathogen (7.0 vs. 5.0 days,  $p < 0.01$ ), and tended to need hospitalization more often than those with another diarrheal pathogen (17.8% vs. 12.3%,  $p > 0.05$ ). At follow-up, death within 60 days had occurred in 7 (4.6%) of 152 cases with *Cryptosporidium* compared with 45 (3.6%) of 1,267 cases without *Cryptosporidium* ( $p > 0.05$ ). Cases from homes that used rainwater as their main source of drinking water had lower odds of *Cryptosporidium*

infection than cases who used other sources (Odds Ratio = 0.58; 95% CI: 0.40-0.85). Cryptosporidiosis causes a substantial burden of diarrheal illness in young children in Kenya. An intervention aimed at reducing the burden of this pathogen is warranted, such as ceramic filtration of household drinking water.

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### SHORT- AND LONG-TERM EFFECTS OF DIARRHEA ON WEIGHT AND LENGTH IN EARLY CHILDHOOD

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The short-term effect of diarrhea on weight faltering is well-accepted, but the long-term effects of diarrhea on both weight and length are less clear. Using data from seven cohort studies, we studied the lagged effect of diarrhea on weight and length in the first two years of life. Our analysis included 1,202 children with 741,846 child-days of diarrhea surveillance and 21,915 length and weight measurements. Using this large, multi-site dataset, we have calculated the effect of experiencing at least one day of diarrhea each month during different time periods on length at 18 months of age. The cumulative effect of monthly diarrhea on length at 18 months of age was -0.58 cm (95% CI -1.11, -0.05). Diarrhea in each of the first 12 months of life was associated with 0.64 cm less attained length (95% CI -1.2, -0.08) when compared with children with no diarrhea during the entire period. There was no statistically significant effect of diarrhea in a specific month of age on length at 18 months of age. Diarrhea in the 30 days prior to the anthropometric measurement was consistently associated with lower weight at most ages, although the association in the early months (1, 2, 3, and 5) was not significant. There was little indication of a lagged short-term effect of diarrhea on length. In this large, multi-site dataset that included frequent anthropometry measurements and complete diarrhea histories, diarrhea had small, but measurable, long-term effects on linear growth when burdens were high. These findings support a focus on prevention of diarrhea as part of an overall public health strategy to improve child health and nutrition; however, other factors, such as dietary sufficiency, may be more important to overall linear growth.

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### LESSONS LEARNED FROM A SECULAR TREND OF DIARRHEAL DISEASES, RISK FACTORS AND NUTRITIONAL STATUS IN COHORT STUDIES OF CHILDREN IN NORTHEAST OF BRAZIL

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Diarrheal diseases and its associated malnutrition are common causes of childhood morbidity and mortality in developing countries. This study was undertaken to describe changes over time in the incidence of diarrheal diseases, risk factors and prevalence of malnutrition among children in an urban Brazilian community from 1989 to 2012. A secondary aim was to examine the number of episodes of acute diarrhea (AD, duration 13 days) episodes of diarrhea and its impact on children nutritional status. We conducted approximately 12-year (Aug-89 to Mar-00) and 2-year (Jun-10 to Mar-12) cohort studies of 415 and 233 children, respectively,

from a Brazilian urban community who were followed from birth. Data were collected on demography, twice weekly surveillance for diarrhea, risk factors and anthropometry. We observed a decline on both episodes and days/child-year over 23 years of follow-up and were correlated with improvements in z-scores nutritional status. The number of AD episodes ( $\geq 3$  AD episodes) were associated with an increased risk for ProD as well as PD episodes with nutritional consequences. The major protective factors associated with decline diarrheal diseases were increased years of mother education, increased days of exclusive or total breastfeeding practice and better sanitation, especial increased proportion of flush to piped sewer system. Other beneficial factors were greater immunization average coverage for measles and introduction of rotavirus vaccination, better living condition and household income. These results demonstrate marked changes over time in the diarrheal diseases and nutritional status. Number of AD episodes ( $\geq 3$  AD episodes) also pose increased risk for significant morbidity and identifies children at risk for ProD and PD episodes and the consequences for the vicious cycle of diarrhea and malnutrition, as well as the chronic process of enteropathy. Further studies are needed to understand the etiology, risk factors and pathophysiology of AD episodes burden.

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### DEMOGRAPHICS, RESISTANCE PATTERNS AND CLINICAL CHARACTERISTICS OF PATIENTS WITH DIARRHEA AND NON-TYPHOIDAL SALMONELLA DETECTED IN STOOL PRESENTING TO A DIARRHEAL HOSPITAL IN DHAKA, BANGLADESH

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Non-typhoidal *Salmonella* (NTS) is a cause of gastroenteritis worldwide, with high rates of invasive disease in sub-Saharan Africa. Data on NTS infection in South Asia are sparse. We used data gathered prospectively from 1996 to 2011 as part of a surveillance system at the Dhaka Hospital of icddr,b, in which every 50th patient undergoes microbiologic evaluation of stool and is queried regarding demographics and clinical manifestations. We identified 468 diarrheal patients in whom NTS were detected in stool, representing 1.3% of all patients surveyed. Over the study period, the frequency of stools positive for NTS decreased (1996-2000, 1.6%; compared to 2007-11, 0.9%;  $P < 0.0001$ ). Highest rates of detection of NTS occurred in the monsoon season (1.5-2.1% of patients, July-Sept), with the lowest rates in the cooler dry season (0.6-0.9%, Jan-Apr). About 47% (219) of total cases of NTS were in children <5 years of age, though when expressed as % of patients, those  $\geq 55$  years had the highest rate of NTS isolated (2.2%). Over the study period, rates of resistance to ampicillin, chloramphenicol, TMP/SMX, and ceftriaxone decreased, while rates of decreased susceptibility to ciprofloxacin increased. Among 345 patients with NTS as the sole pathogen identified in stool, 93% reported watery stool, 46% >10 stools in the 24 hours prior to presentation, 78% vomiting, and 48% abdominal pain. Of those <5 years of age ( $n=162$ ), 41% were stunted and 48% underweight. 16% of patients presented with severe dehydration, 54% with lethargy or drowsiness, and 10% with a recorded presentation temperature of  $>37.8^{\circ}\text{C}$ . 23% required IV rehydration and <1% died ( $n=2$ ). Routine evaluation for bacteremia is not part of the surveillance system, but NTS is not a common cause of bacteremia at the icddr,b (0.6% of all positive blood cultures in 2009-2011). In conclusion, NTS is isolated as the sole pathogen in stool cultures of cases of gastroenteritis requiring hospital care in Dhaka, Bangladesh, with a high burden in malnourished young children and the elderly, but is not a common cause of bacteremia.

## GENES OF THE ANGIOGENESIS PATHWAY ARE ASSOCIATED WITH DEVELOPMENT OF HYDROCELE IN LYMPHATIC FILARIASIS - A CANDIDATE-GENE ANALYSIS

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Of the ~1 billion people at risk in endemic countries, >120 million people are estimated to be infected with *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*, the causative agents of lymphatic filariasis (LF). Studies have shown that susceptibility to infection, parasite load and lymphatic pathology cluster in families but only a few studies have looked for genes associated with LF and its different clinical presentations, mainly lymphedema and hydrocele. To elucidate the genetic basis of- and possible genetic markers for hydrocele development, which affects 50% of infected men, we performed a candidate gene analysis with 850 *Wuchereria bancrofti* infected volunteers by genotyping 48 single nucleotide polymorphisms (SNPs) in 32 angiogenesis genes by MassARRAY. We found variants in four genes of the angiogenesis pathway significantly associated with hydrocele patients compared to infected individuals without pathology. A SNP in the coding region of the Endothelin-1 gene was associated with pathology (rs5370,  $P=0.015$ ) and formed a haplotype with a SNP in the promoter region (rs1800541, corrected haplotype  $P=0.020$ ). An intron SNP of Caveolin-1 (rs4730748,  $P=0.007$ ) was associated with hydrocele and formed a haplotype with a second intron SNP (rs926198, corrected haplotype  $P=0.002$ ). A SNP in the collagen type 1 alpha 1 gene (COL1A1) and the matrix metalloproteinase 2 gene (MMP-2) were also associated with hydrocele pathology (COL1A1  $P=0.028$ ; MMP-2  $P=0.042$ ). To functionally characterize the SNPs, plasma from the study participants has been measured for systemic levels of the respective proteins to be correlated with genotype and expected phenotypes. Our results underscore the fact that hydrocele pathology due to LF has a complex genetic basis, i.e. it is multi-factorial. Further characterization of these 4 angiogenesis pathway SNPs may result in a predictive screen to help physicians prevent pathology development or lead to therapies to ameliorate existing pathology.

## IS THERE BLINDING ONCHOCERCIASIS IN UGANDA? EVIDENCE FROM PADER DISTRICT IN NORTHERN UGANDA

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Onchocerciasis caused by a filarial nematode *Onchocerca volvulus* is transmitted by female black flies of Genus *Simulium* (Diptera: Simuliidae) which breeds in fast flowing rivers. The most common manifestations of onchocerciasis in Uganda are nodules and onchodermatitis. There is virtually no information on ocular lesions. In 2010, a short study on *Simulium damnosum* transmitted onchocerciasis was conducted in Pader district, Northern Uganda. One of the study's main objectives was to determine whether onchocerciasis in this region is the blinding type, with a view to determining the appropriate treatment strategy. A total of 675 persons from 13 randomly selected parishes were examined for clinical, parasitological and ocular manifestations of onchocerciasis. The prevalence of microfilaria in skin snips was 28%, while that of nodules and onchodermatitis was 30% and 29% respectively. The most common

skin lesion was Chronic Papular Onchodermatitis (17.5%). However, the prevalence of microfilariae of *O. volvulus* in the anterior chamber of the eye and reversible ocular lesions was 4% each. The reversible ocular lesions of onchocerciasis observed were punctate keratitis stage B (0.1%), punctate keratitis stage D (0.1%) and punctate keratitis stage E (3.7%). On the other hand, 16.1% of the individuals examined had irreversible ocular lesions attributed mainly to Optic atrophy (6.4%) and sclerosing keratitis (5.2%). Visual impairment was detected in 29.2% of those examined and most were due to cataracts (27%) and Optic Atrophy (26%). There was significant association between irreversible onchocercal lesions and visual loss ( $p < 0.0001$ ) and irreversible lesions and nodules ( $p < 0.0001$ ). This study confirms for the first time the occurrence of blinding strains of *O. volvulus* in northern Uganda. It is recommended that treatment [Mass Drug Administration (MDA)] be administered semi-annually rather than once per year, the current norm. Vector control measures should be instituted to reduce the burden of onchocerciasis, especially visual impairment leading to blindness.

## MASS DRUG ADMINISTRATION: POTENTIAL FOR REVERSING SUBCLINICAL LYMPHATIC PATHOLOGY IN WUCHERERIA BANCROFTI INFECTION

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The occurrence and importance of childhood lymphatic pathology in filariasis endemic areas is being increasingly recognized. We examined the impact of treatment with Albendazole and DEC, drugs used in Mass Drug Administration (MDA), on lymphatic pathology in 100 *Wuchereria bancrofti* infected symptomatic and asymptomatic children (5-18 years) from filarial endemic villages of Odisha, India. 52 children were asymptomatic while the others had early lymphedema, hydrocele or lymphangitis. After clinical examination and screening for microfilaremia and antigenemia (Og4C3 antigen) they underwent lymphoscintigraphy using Tc99 sulphur colloid to detect lymphatic pathology in lower limbs and ultrasonography for detection of adult worms. Lymphatic pathology (visualization of lymphatic tract, non visualization of inguinal nodes, and low tracer uptake ratio at inguinal level at unit time and presence of collateral lymphatic channels in contra lateral limb) was identified in 63 children of whom 29 were asymptomatic. Adult worms were detected in 9 children. Children were randomised to receive either annual (n=49) or biannual (n=51) dose of DEC (6mg/kg) and Albendazole (400 mg) and followed up at six monthly intervals when investigations were repeated. Follow up of 50, 31 & 20 children at 6, 12 & 18 months respectively has been completed. We observed a clinically significant impact on the lymphatic pathology even as early as 6 months, with improvement in the appearance of lymphatics, loss of co-laterals and flow improvement. Improvement in lymphatic flow was observed in 21/26, 16/18, & 9/10 children at 6, 12 & 18 months respectively; while complete reversals were observed in three children at the 12 month follow up. Our findings while demonstrating that treatment can reverse pathology of both early symptomatic and asymptomatic children reiterate the importance of treating early, when there is the possibility that pathology can be reversed. They also provide a powerful message that could be utilized to strengthen the MDA advocacy efforts of the elimination programme.

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### SHORTENING THE TIMEFRAME AND DOSAGE OF ANTI-WOLBACHIA THERAPY: DOXYCYCLINE ALONE VERSUS DOXYCYCLINE PLUS RIFAMPICIN IN THEIR EFFICACY AGAINST LYMPHATIC FILARIASIS; A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL

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The current standard for the treatment of lymphatic filariasis (LF) using antibiotics to target *Wolbachia* endosymbionts is doxycycline (DOX) 200mg/d for 4 weeks, with high macrofilaricidal activity. To test the efficacy of a reduced dosage of DOX to 100mg or shortening the treatment period using a combination of DOX 200mg and rifampicin (RIM) 10 mg/kg/d, a field trial was conducted as part of the A-WOL program. In a Ghanaian area endemic for LF, men were screened by ultrasound for live adult worms (filarial dance sign, FDS). 301 men with FDS were randomized into seven treatment arms (4 weeks (w) DOX 200mg/d, 5w DOX 100mg/d, 4w DOX 100mg/d, 3w DOX/RIM, 2w DOX/RIM, 10 days DOX/RIM, 5w placebo). 254 men completed the treatment per protocol. After four months the participants received ivermectin plus albendazole. Outcome measures were absence of FDS (macrofilaricidal effect) as well as absence of microfilariae (Mf; long-term sterilizing effect). The 5 weeks DOX 100mg/d group showed 75%, 89% and 88% macrofilaricidal activity (absence of FDS) at 12, 18 and 24 months, respectively, compared to 71%, 76% and 83% in the standard and 37%, 36% and 48% in the placebo groups, i.e. showing superiority to placebo and equivalence to standard therapy at all time points. 86% macrofilaricidal activity was also reached with 4w DOX 100mg/d at 18 months. Compared to placebo, significantly fewer men treated with DOX alone or 3w DOX/RIM were Mf positive at 12 months. In the 4w and 5w DOX 100mg/d arms, this effect persisted until month 18 and 24, respectively. The reduction of the DOX dosage from 200mg/d to 100 mg/d (4 or 5 weeks) resulted in >85% macrofilaricidal activity and a long-term sterilizing effect at 18 months, and therefore should be recommended for treatment of LF. This dosage and regime is now equivalent to that recommended for malaria prophylaxis in travellers and for acne. Depending on the favored outcome, a shortened treatment period with the combination of DOX and RIM may be chosen to achieve a long-term sterilizing effect for 12 months.

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### ORAL ADMINISTRATION OF A NEW FORMULATION OF FLUBENDAZOLE SHOWS EFFICACY IN THE FILARIAL INFECTION *LITOMOSOIDES SIGMODONTIS*

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Flubendazole is a macrofilaricidal and has been shown to be active in many species including humans. As a very insoluble chemical it has been a challenge to develop formulations that achieve efficacious plasma levels by oral administration. An orally administered amorphous solid dispersion

form of this benzimidazole was seen to have a significant effect on the viability, worm load and pathology on a natural infection of jirds with *Litomosoides sigmodontis*. Initially plasma levels of flubendazole were measured in jirds administered 2, 5, and 20 mg/kg. Peak plasma level was at 2 hours and was nearly cleared by 8 hours. Jirds infected with *L. sigmodontis* were administered 0, 2, 6, 20 mg/kg of flubendazole orally once a day for 5 days and then sacrificed at 8 weeks. Tissue pathology was scored, identifiable worms from the peritoneal and pleural cavities isolated and counted, and MTT assay carried out on the isolated worms. A dose response for tissue pathology, worm burden and MTT assay were observed. This study represents one of the first descriptions of the filariacidal activity of flubendazole when administered orally. The possibilities this approach to the MDA for filarial control and eradication programs will be discussed.

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### ANTI-WOLBACHIA CONSORTIUM (A•WOL) DRUG DISCOVERY - SCREENING OF FOCUSED ANTI-INFECTIVE LIBRARIES FOR NOVEL COMPOUNDS WITH EFFICACY AGAINST *WOLBACHIA* ENDOSYMBIONTS OF FILARIAL NEMATODES

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Filarial nematodes are an important group of pathogens infecting 150 million people throughout the tropics with over 1.5 billion at risk. Control of filariasis currently relies on mass drug administration (MDA) programmes using drugs which principally target the microfilarial life-cycle stage. Antibiotic targeting of filarial *Wolbachia*, an essential bacterial symbiont, provides a novel treatment with macrofilaricidal activity. In order to turn this therapy into a public health tool suitable for filariasis control programmes, the Anti-*Wolbachia* Consortium (A•WOL) utilises *in vitro* cell and nematode assays, followed by secondary *in vivo* assays, to screen both focused and diversity compound libraries against *Wolbachia*. Here we describe the screening of 5399 compounds, from five focused anti-infective chemical libraries, in a *Wolbachia* cell-based assay. We have identified 263 hits, with 117 compounds showing improved efficacy over doxycycline against *Wolbachia*. Hit compounds also show activity against nematode *Wolbachia in vitro* without exhibiting direct anti-nematode activity. Based on our hit criteria, 104 compounds have progressed down the screening funnel and been screened in a *Litomosoides sigmodontis* mouse model, where encouragingly, a number show equivalent or improved efficacy compared with doxycycline against *Wolbachia in vivo*.

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### SUCCESSFULLY MANAGING SERIOUS ADVERSE EVENTS (SAES): LESSONS LEARNED FROM NEPAL'S LYMPHATIC FILARIASIS ELIMINATION PROGRAM

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Sixty of Nepal's 75 districts are considered endemic for lymphatic filariasis (LF), putting an estimated 25 million people at risk for contracting LF. In 2002, the Government of Nepal (GoN) formulated a National Plan of Action (2003-2015) for elimination of LF by 2018. Forty six LF-endemic districts have begun LF control programs through mass drug administration (MDA) of diethylcarbamazine and albendazole. These campaigns, targeting entire communities, are implemented by GoN with support from international donors. GoN started LF MDA as a pilot in one district in 2003 and scaled it up to 5 districts in 2006. Among



the Serious Adverse Events (SAEs) reported during this MDA were 15 deaths. Though an independent investigation team confirmed that none of these deaths were associated with MDA, the deaths caused deep concern in the community. Following this event, GoN focused on coordination and advocacy to establish community trust in MDA, and there was no MDA during 2007. With proper advocacy and coordination, the LF MDA restarted in 2008 and was scaled up to 36 districts in 2011. About 250 SAEs including 8 deaths were reported during the 2011 LF MDA. Widespread negative media coverage created fear about MDA within communities, even though an investigation team did not find an association between MDA and deaths. The team recommended a number of actions for future MDAs to minimize and properly manage SAEs. Based on these recommendations, GoN revised its guidelines and emphasized obtaining political commitment and engaging the media and communities for increased advocacy. Efforts were made to increase coordination among health providers, set up response teams in health centers, establish referrals for possible SAEs, and ensure timely response to SAEs. This approach enabled GoN to gain back community trust in the program and there was no interruption in the 2012 LF MDA. Similar efforts by governments and entities implementing MDA in settings beyond Nepal can ensure that SAEs do not hamper program implementation and successes achieved.

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### ORIGIN, DIVERSITY AND MOLECULAR CHARACTERIZATION OF A NOVEL PROTEIN-CODING GENE FAMILY WITH SIMILAR SIGNAL SEQUENCE IN *SCHISTOSOMA JAPONICUM*

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Evolution of novel protein-coding genes is the bedrock of adaptive evolution and acquisition of novel molecular functions. Recently, we identified a novel protein-coding gene family with similar promoter region and signal sequence from *S. japonicum* using signal sequence trap (SST) that isolates secreted and membrane bound molecules. Here, we adopted an integrated approach utilizing bioinformatics and molecular tools to delineate the underlying mechanisms of this phenomenon; and performed functional characterization of the candidate genes, which we found exclusively expressed in *S. japonicum*. Our analyses and southern hybridization results suggest disperse gene duplication mechanism as a product of DNA-level recombination, mediated by repetitive elements (REs) as inferred from the observed flanking REs (RTE-SJ and Perere classes) around the duplicated gene loci. To investigate the possibility of selective pressure acting on the evolving genes, we sequenced the coding sequences of the genes from the genomic DNA of some strains of *S. japonicum*, and as expected, identified a significant balancing selection pressure, especially on the putative functional domains of the candidate genes. In addition, a stepwise pathway towards functional selection by alternative splicing was established. While no ancestral homolog was found in other organisms, 3D structure screens revealed similar folding pattern with SEA and SEA-like modules common among proteins in carbohydrate rich environment. We will present data on the molecular characterization and immunogenicity of these candidates from our functional assays and protective effect trials. The role of REs as major mediators of DNA-level recombination leading to dispersive duplication is once more highlighted with evidence from our analyses. Our findings contribute to the growing evidence of the role of REs in the generation of evolutionary novelties in organisms' genomes. *S. japonicum* has wide

range of mammalian hosts and produces more severe hepatic pathology than *S. mansoni*. Evolutionary novelties after its divergence could account for these distinctive characteristics.

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### NEW VIEWS OF SCHISTOSOME DIVERSITY

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The *Schistosomatidae* comprises about 100 species of blood-inhabiting digenetic trematodes with major medical and veterinary significance. Although the family is comprised of 4 subfamilies, one of them, *Griphobilharzinae*, is represented by only one species from a crocodile that, as shown by molecular studies, is a member of a different family, the *Spirorchidae*. This is supported by the recent finding of spirorchids from Nepalese snails that cluster with *Griphobilharzia*. As presently known, schistosomes are exclusively parasites of birds and mammals. For *Schistosoma*, relationships among species have been relatively well-defined, including the realization that *Orientobilharzia* clusters within *Schistosoma*, suggesting name changes for the latter genus are in order. New molecular data for schistosomes from elephants (*Bivitellobilharzia*) confirm this is a monophyletic genus consisting of two species, one of which is also reported for the first time from the Asian rhinoceros. Our studies indicate that the diversity of avian schistosomes, that collectively cause swimmer's itch, is considerable. Even in relatively arid New Mexico, 10 species of avian schistosomes have been recovered. Increasingly, molecular signatures can be provided enabling the species responsible for dermatitis outbreaks to be identified. One new genus of avian schistosomes has recently been erected, and another is in progress. Additional likely new genera await further study as we have found both cercariae and adult worms that do not match any known schistosome sequences. The derived clade of avian schistosomes reveals evidence of extensive host switching, particularly with respect to the snail host. For example, schistosomes transmitted through freshwater snails can group closely with those transmitted in marine snails. Additional studies are needed to gain a more complete overall understanding of schistosome diversity before it is lost, and to better resolve the deeper phylogenetic relationships among schistosome genera.

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### COUNTRY MOUSE-CITY MOUSE: WHEN SCHISTOSOMES COME TO TOWN

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Although often considered a rural disease, schistosomiasis is frequently being observed in urban settings due to internal migration and strained sanitation infrastructures. The neighborhood of Sao Bartolomeu in Salvador, Bahia, Brazil is a focus of urban schistosomiasis. It is a largely poor community divided into 6 microareas (MA) straddling the Cobre River. Here we examined 1213 of all 1508 individuals from three geographically separated MAs for helminth infection. *Schistosoma mansoni* prevalence rates of 21.9%, 24.6%, and 27.6% were observed in MAs 1, 3, and 6, respectively. Eggs were collected by selective sieving from whole stools of 308 infected individuals. Each sample represented the *S. mansoni* population within each host (intrapopulation). DNA extracted from the egg samples was genotyped for 15 microsatellite loci, and genetic differentiation analyses were performed based on population allele frequencies. Only MAs 3 & 6 have been analyzed so far. Moderate differentiation (mean pairwise Jost's D values 0.053 and 0.043, respectively) was observed within intrapopulations. Similarly, D = 0.049 between the 2 MAs. This profile of an urban focus contrasted with those of two rural villages in Bahia separated by 8 km along the Jiquiriçá River.

Here the prevalence was greater at 40%. The pairwise infrapopulation  $D_s$  were also greater at 0.08 and 0.12 within the villages and 0.134 between villages. When combined into component populations,  $D$  between rural populations was 0.046 compared to 0.004 between the component populations at the urban sites. Slightly lower genetic diversity was seen in urban vs. rural populations (mean effective allele numbers = 3.62 and 3.78 across loci). If infection were due to only in-migration from the many different areas of Bahia represented in São Bartolomeu, the differentiation and diversity might be expected to be greater than that of a single rural area. This may suggest that the primary transmission here is occurring locally.

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### EFFECTS OF PRAZIQUANTEL ON GENE EXPRESSION OF MALE AND FEMALE *SCHISTOSOMA MANSONI* ADULT WORMS IN CULTURE

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*Schistosoma mansoni* is one of the agents of schistosomiasis, a chronic and debilitating disease. Until now, there is no effective vaccine available for schistosomiasis and praziquantel (PZQ) is the drug of choice for large-scale treatment; unfortunately, the mechanisms of action of PZQ so far are not fully understood. We used a microarray platform to measure large-scale gene expression of adult worms that were submitted to and survived PZQ treatment. SAM one-class statistical test was applied and genes were considered as significantly differentially expressed at  $q$ -value  $\leq 0.05$ . Our results show gene expression of female adult worms was dependent on their male-pairing status when treated with PZQ. Noteworthy, separated females had almost 3 times more differentially expressed genes (1,434) than paired females (486), which could be an indication of why paired females are more sensitive to PZQ when compared to separated females, and an indication of alternative networks and escape mechanisms of female worms. Our analysis showed 219 genes commonly affected by PZQ in both separated and paired females; 96% of these genes showed an inverted expression pattern that was dependent on the pairing status of the females. We also observed differences in gene expression related to the gender of the adult parasite, when comparing paired male and female worms exposed to PZQ. We found that 48 genes were commonly affected by PZQ in paired male and female adult worms, when compared to their non-treated respective controls. Ingenuity Pathway Analysis (IPA) and Text Mining software were used for functional analyses, identifying gene networks that were significantly enriched with differentially expressed *S. mansoni* genes; among them, some had human homologs that are known targets of other drugs, pointing to possible new parasite targets for a combined treatment regimen for schistosomiasis. These results provide important information to understand the differences related to *S. mansoni* susceptibility to PZQ and therefore differences in the possible mechanisms of action of the drug.

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### CHEMICAL AND GENETIC BIOLOGY OF *SCHISTOSOMA MANSONI* PHOSPHODIESTERASE 4 - A POTENTIAL THERAPEUTIC TARGET

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Schistosomiasis is an infectious tropical disease caused by the *Schistosoma* blood fluke. Treatment and control of this disease relies on just one drug and there is a growing concern over the possible emergence of drug resistance. To identify new drug targets we employed a qualitative, phenotypic screening platform evaluating *S. mansoni* parasites against a collection of phosphodiesterase (PDE) inhibitors provided by Anacor Pharmaceuticals of Palo Alto CA. We found that a series of cyclic nucleotide phosphodiesterase 4 (PDE4) inhibitors induce parasite hypermotility and eventually death. PDE4 catalyzes the hydrolysis of the intracellular second messenger, cyclic AMP, regulating its concentration in cells. PDE4 inhibitors are under pre-clinical and clinical evaluation to treat a range of disorders, including other tropical diseases. In the free living nematode, *Caenorhabditis elegans*, the same PDE4 inhibitors active against *S. mansoni* also cause hypermotility. Furthermore, a hypermotile phenotype is seen in *C. elegans* mutants deficient in the orthologous pde4 gene (R153.1). The *C. elegans* gene is localized to synapses—a location consistent with a function in neural cAMP signaling pathways that regulate locomotion. These findings set the stage for (i) inquiring whether the hypermotility of *C. elegans* induced by the Anacor benzoxaborole compounds is indeed caused by inhibition of PDE4 and (ii) whether the human and *S. mansoni* orthologs of *C. elegans* pde4 can functionally substitute for R153.1 mutants. Accordingly, by “humanizing” and “schistosomizing” the *C. elegans* pde4, we can take advantage of the amenability of *C. elegans* for genetic analyses and drug screens. We will use *C. elegans* as a surrogate worm to select for compounds that inhibit *S. mansoni* PDE4 but not the human counterpart. Data arising from the chemical screens and genetic experimental approaches will be presented.

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### INTERACTION WITH THE HEMOGLOBIN DEGRADATION PATHWAY OF *SCHISTOSOMA MANSONI* AS A RATIONALE FOR ANTISCHISTOSOMAL DRUG DISCOVERY

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The common bloodfeeding characteristic of schistosomes and *Plasmodia* has led to studies with antimalarial drugs against schistosomes in recent years. Amongst different chemical structures, two qualified as leads for antischistosomal drug discovery: mefloquine, a 4-quinolinemethanol, and the artemisinins with their distinct peroxidic scaffold. We will present our work with a group of peroxides, the 3-alkoxy-1,2-dioxolanes as well as mefloquine related compounds belonging to 4-pyridinemethanols, 9-phenanthrenmethanols, and related 4-quinolinemethanols. Additionally we will describe the roles of iron and the peroxidic core in drug activity. All candidates were tested against juvenile and adult stages of *Schistosoma mansoni* *in vitro*. Successful candidates were followed up *in vivo* on *S. mansoni* infections in mice. Three dioxolanes showed promising *in vitro* activity on both stages ( $IC_{50}$ 's  $\leq 20.1$   $\mu$ M). However, only moderate, non-significant activity was observed *in vivo*. Two dioxolanes showed high *in vitro* activity against *E. caproni*, a non-blood-feeding intestinal fluke, and additional iron sources did not alter activity on schistosomes, supporting an iron-independent mode of activation. Non-peroxidic analogues lacked activity against both parasites, underlining the necessity of a peroxide

functional group. Amongst nine selected mefloquine-related compounds, two 4-quinolinemethanols (WR7573, WR7930) showed significantly lower  $IC_{50}$ s ( $< 3.5 \mu\text{M}$ ) than mefloquine ( $11.4 \mu\text{M}$ ) against schistosomes *in vitro*. Mefloquine and WR7930 showed significantly decreased  $IC_{50}$ s when incubated in the presence of hemoglobin. High efficacy was observed for enpiroline and WR7930 against adult *S. mansoni* in mice. Enpiroline, WR7930 and mefloquine also showed high *in vivo* activities against *S. haematobium*. In conclusion, among the different arylmethanols tested the 4-quinolinemethanols reveal the greatest potential as starting point for antischistosomal discovery. Regarding the dioxolanes low *in vivo* activities would need to be overcome to identify an antischistosomal lead candidate.

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### PRAZIQUANTEL RESISTANCE IS EXPERIMENTALLY INDUCED IN *SCHISTOSOMA JAPONICUM*

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Praziquantel is currently the only drug of choice for treatment of human schistosomiasis. It is worrying about that following subcurative treatment, drug resistance may emerge. The purpose of the present study was to investigate the possibility of the emergence of praziquantel resistance in *Schistosoma japonicum* in Mainland China under drug pressure. Following 8-passage subcurative selection, treatment with praziquantel at single oral doses of 300 and 600 mg/kg achieved worm burden reductions of 32.6% and 68.1%, 45.7% and 61.9%, respectively in Jiangsu and Hunan inducing isolates of *S. japonicum*, whereas being 71.5% and 97.4%, 70.8% and 97.5% in the corresponding field isolates without drug selection. It is concluded that *S. japonicum* is able to develop resistance to praziquantel under subcurative drug selection.

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### EXTENSIVE DIVERSITY OF *TRYPANOSOMA CRUZI* DISCRETE TYPING UNITS CIRCULATING IN *TRITATOMA DIMIDIATA* FROM CENTRAL VERACRUZ, MEXICO

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Chagas disease (or American trypanosomiasis) is a parasitic disease of major public health importance, caused by *Trypanosoma cruzi*, which presents extensive genetic diversity. The parasite has been classified into six lineages or discrete typing units (TcI to TcVI) and we performed here the molecular characterization of the strains present in *Tritatoma dimidiata*, the main vector in central Veracruz, Mexico. Unexpectedly, TcI only represented 9/33 strains identified (27%), and we reported for the first time the presence of TcII, TcIII, TcIV and TcV strains in Mexico, at a relatively high frequency (13-27% each). Our observations indicate a much greater diversity of *T. cruzi* DTUs than previously estimated in at least part of Mexico. These results have important implications for the understanding of the phylogeography of *T. cruzi* DTUs and the epidemiology of Chagas disease in North America.

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### LATENT INFECTION WITH *LEISHMANIA DONOVANI* IN HIGHLY ENDEMIC VILLAGES IN BIHAR, INDIA

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In Visceral Leishmaniasis (VL) asymptotically infected persons usually outnumber clinically apparent cases by a ratio of 4-10 to 1. Though little is known about their infectiousness, these latent carriers present a potential challenge for the VL elimination effort. The fact that current diagnostic tests have been validated to detect clinical VL cases but not to detect asymptomatic infections further complicates matters. We report population-based data from a cohort study in a highly endemic area in Bihar, India, describing patterns of markers of infection in relation to age and to other known risk factors for clinical VL. We selected eleven highly endemic villages that were part of a larger study in which annual household surveys are being conducted and information on VL and risk factors for VL has been collected. Two rounds of sero surveys were conducted in which blood samples on filter paper were collected for all those aged 2 years and above who were present at the time of both survey rounds and provided informed consent. Samples were tested by DAT and rK39 ELISA. We enrolled 11,399 persons, 8% of whom were reactive to DAT and 7% to rK39 during the first survey round. Of the 9,919 initially sero negatives, 363 (3.7%) had converted to sero positive one year later. Fifty three percent of initially rK39 positives and 25% of initially DAT positives had reconverted to negative at the time of the second survey round. Whereas clinical VL occurs mainly among young adults, seropositivity and sero conversion were more common with advancing age and were not associated with known risk factors for clinical VL. The age pattern observed in seropositivity and seroconversion could be explained by a boosting effect upon repeated exposure to the parasite. More work is needed to elucidate the significance of serological markers of infection and the role of latent carriership of *L. donovani* in VL transmission.

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### A WHOLE BLOOD IFN- $\gamma$ /IL-10 RELEASE ASSAY AS A MARKER OF ASYMPTOMATIC INFECTION AND DISEASE STATUS IN HUMAN VISCERAL LEISHMANIASIS

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In endemic area, a large number of exposed individual are able to mount a protective cellular immune response against *Leishmania* and either eliminate infection or remain asymptomatic carriers. Leishmanin skin test (LST) or serological methods have been used to document incident infection, but their value as marker has not been firmly established so far. However, a valid marker of infection is crucial for intervention studies on vaccine or vector control and for monitoring of ongoing transmission in endemic area. We investigated the soluble leishmania antigen (SLA) based whole blood assay on a larger cohort of subjects, to detect IFN- $\gamma$ , TNF- $\alpha$  and IL-10 released cytokines and aimed to correlate with the marker of asymptomatic infection and disease status of infected individuals. We employed the whole blood assay to evaluate IFN- $\gamma$ , TNF- $\alpha$  and IL-10 production in 35 patients with active visceral leishmaniasis (VL), 54 patients with cured VL, 27 patients with other diseases and 52

Non-Endemic Healthy Controls (NEHC). We also tested the assay on 147 Endemic Healthy Controls (EHC), all close contacts of VL cases having a high probability of *L. donovani* infection, and correlated their cellular response with their serological antibody titers against *L. donovani* and *Phlebotomus argentipes* saliva. The whole blood IFN- $\gamma$  release assay had a sensitivity of 85.2% (95% CI 73.4 - 92.3) in cured VL patients to detect the cellular immune response and a specificity of 100% (95% CI 93.1 - 100.0) in NEHC. The assay detected IFN- $\gamma$  release in 24% of the EHCs, and these individuals also had elevated titers of anti-saliva antibodies, consistent with their having had a higher risk of exposure to an infected sand fly bite. Only the active VL patients produced IL-10, which can therefore be assayed in conjunction with IFN- $\gamma$  to distinguish active cases from clinically exposed, immune individuals. The findings strongly reinforce the utility of the SLA based whole blood assay for the detection of IFN- $\gamma$  production by *L. donovani* infected, asymptomatic individuals, and for detection of IL-10 secretion as the signature cytokine distinguishing active VL from cured or asymptomatic cases.

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#### CHAGAS DISEASE AMONG LATIN AMERICA IMMIGRANTS EVALUATED IN A TROPICAL MEDICINE CLINIC IN BRONX, NEW YORK

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Due to increase awareness, immigration and screening of the blood supply Chagas disease is an important "emerging" infection and public health issue in the United States (US). This study describes a 7 year (2005-2012) experience with Chagas disease in a tropical medicine clinic in NYC. Patients were referred from the Blood Center or from a primary care physician. Antibodies were detected with ELISA and were confirmed with IFA performed at CDC. Baseline blood counts, electrocardiogram (EKG) and echocardiogram (echo) were performed. Twenty-one patients (12 male [57%]), mean age of 45 $\pm$ 17 years were evaluated. Patients were from Mexico (6), El Salvador (6), Ecuador (3), Argentina (1), Honduras (1), Bolivia (1) and 3 were born in the US to immigrant mothers. Mean time from immigration was 19 $\pm$ 9 years. History of living in a mud house was recalled by 7(44%) patients. Twelve patients (57%) were diagnosed as a result of blood transfusion screening. Abnormal EKG findings were observed in 7 patients, including: sinus bradycardia, first degree AV block, right bundle branch block. Six patients had abnormal echo findings: left ventricle apical aneurysm, wall motion abnormalities, diastolic dysfunction and chambers dilatation. Four (20%) patients, with a mean age of 67 $\pm$ 9 years, had advanced cardiac disease; one patient required a transplant. Ten patients were asymptomatic (mean age 4 $\pm$ 14 years); 4/6 asymptomatic patients had normal EKG and echo. Six patients (mean age 32 $\pm$ 8 years) were treated, 4 with nifurtimox and 2 with benznidazole. All patients developed side effects; cutaneous toxicity was associated with benznidazole, leading to discontinuation of drug; and headaches, insomnia, myalgias and gastrointestinal complaints with nifurtimox. Our experience underscores the importance of screening immigrants who are both asymptomatic or have cardiac abnormalities. US-born children who have never visited endemic areas are also at risk of mother to child transmission and should be screened. Physicians in developed countries should become familiar with the diagnosis of this disease.

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#### DRUG DISCOVERY ALGORITHM FOR CUTANEOUS LEISHMANIASIS

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The development of new drugs for cutaneous leishmaniasis has been hampered by lack of disease awareness, funding, and drug discovery models that predict clinical outcome. Cutaneous leishmaniasis is clinically widespread but lacks treatment options that are effective and well tolerated. Because all present drugs have been grandfathered into clinical use, there are no examples of a pre-clinical product evaluation scheme that lead to new candidates for formal development. We have developed a testing strategy that features a gated, resource sparing model that progresses from high-throughput *in vitro* promastigote and axenic amastigote assays to more clinically relevant, comparable mouse *Leishmania* suppression and *Leishmania* cure models that have undergone internal validation and are reproducible. The results of our automated, high-throughput screening of potential drugs *in vitro* against promastigotes was recently published and the complimentary screening against axenic amastigotes is detailed elsewhere. Our process for advancing compounds from hit to lead which includes compound throughput and additional compound activity metrics will be discussed.

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#### USING METABOLOMIC AND PROTEOMIC ANALYSES TO STUDY DRUG RESISTANCE IN *LEISHMANIA INFANTUM*

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Drug resistance in leishmania is extremely prevalent and a huge obstacle to successful treatment of the disease. The mechanisms of resistance are complex and whole genome studies have revealed a huge amount of inter- and intra-species variation. We demonstrate that strains resistant to leishmanicidal drugs exhibit cross tolerance, shown by lack of ROS induction, a lack of membrane potential loss and a lack of DNA laddering, to other leishmanicidal drugs, while retaining susceptibility. Hydrogen sulphide production was analysed and found to be reduced in resistant strains compared to sensitive, which in bacteria was shown to have effects on glutathione reductase and super oxide dismutase expression. Metabolomics is a relatively new tool in the biologist's toolbox, aiming to analyse the small chemical compounds that determine the front end of cell metabolism. By subjecting metabolites from drug sensitive and drug tolerant strains to mass spectrometry and NMR analysis, we highlight changes to the way the parasite's metabolome responds to drugs. These alterations to the response pathways may underlie the selection of cross-tolerant and further multi-drug resistant strains. As many leishmanicidal drugs are targeted towards the mitochondrion of the parasite, we were also interested in characterising quantitatively the mitochondrial proteome in drug sensitive and resistant strains to determine whether the metabolomic changes seen can be explained by differential expression (using RNAseq data) or modifications to these proteins. The new methods for the purification of mitochondria using differential gradients and Zone Free Flow Electrophoresis will be discussed, in addition to the latest results obtained using these methods. By determining the drug combinations which more easily lead to cross tolerance and resistance, we hope to inform the clinical treatment of leishmaniasis and by determining the metabolic mechanisms by which tolerance and cross-resistance occur, we suggest compounds which may ameliorate the action of the drugs.

## NON-SEROLOGICAL DETECTION OF PARASITE BIOMARKERS IN HUMAN BLOOD FOR THE DIAGNOSIS OF CHAGAS DISEASE

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*Trypanosoma cruzi*, a blood borne pathogen, is the etiological agent of Chagas disease in humans. Following an infection, patent parasitemia is detectable in the blood of infected individuals and this phase is termed the acute phase. This is followed by a chronic phase that persists for the life of the individual. During this phase, 20-30% of infected individuals will develop clinical symptoms. Diagnostic assays for Chagas disease detect host anti-*T. cruzi* antibodies as a surrogate marker for infection. However, these assays are not reliable during the early period when infected individuals have not yet sero-converted. Additionally, serological tests are unreliable for determining treatment efficacy as anti-*T. cruzi* antibodies persist for a long time and their levels do not correlate with parasite clearance. To overcome these drawbacks, we envisaged the detection of antigens secreted by parasites, collectively termed as *T. cruzi* Excreted Secreted Antigens (TESA), as a diagnostic for Chagas disease. We utilized *in-vitro* RNA SELEX methods to develop TESA aptamers (short RNA molecules) with the goal of utilizing them as specific ligands in detection assays. The TESA SELEX was performed using cell culture supernatant from *T. cruzi* trypomastigote infected NIH 3T3 cells. Biotinylated monoclonal aptamers were utilized in a modified enzyme linked assay to detect TESA in sera from Chagasic patients. Patient samples were obtained from endemic areas (Goias and Minas Gerais) of Brazil and were clinically classified as acute and chronic infections. Several aptamers screened showed significant binding to serum from infected individuals compared to non-infected endemic controls. These interactions were specific, as scrambled aptamers did not bind to serum from infected individuals. This is the first demonstration of an aptamer based assay that detects a parasite biomarker for the diagnosis of Chagas disease in humans.

## THE TORORO CHILD COHORT: A LONGITUDINAL STUDY OF MALARIA IN THE SETTING OF INSECTICIDE TREATED BEDNETS AND ARTEMISININ-BASED COMBINATION THERAPY

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Many reports have suggested that malaria incidence is decreasing in Africa in the setting of malaria control interventions, but longitudinal studies are lacking. We enrolled a cohort of 100 HIV negative children 6 weeks to 10 months of age in an area of high malaria transmission intensity in Uganda and followed them through 48 months of age. Children were enrolled during 2007-2008, given LLITN at enrollment, and received all care at a study clinic, including ACTs for episodes of malaria and routine monthly blood smears. Malaria incidence was measured using passive surveillance with incident malaria defined as a fever and positive thick blood smear in the absence of malaria treatment in the prior 14 days. Asymptomatic parasitemia was defined as a positive smear in the absence of fever and not followed by malaria within 7 days. We calculated the relative risk of incident malaria by age, calendar time and season using generalized estimating equations with robust standard errors. 100 children were

enrolled (mean age at enrollment 5.52 months) of whom 79 reached 48 months of age; children were followed for a median of 3.46 years. A total of 1633 incident episodes of malaria were observed in this cohort, with a median incidence of 5.32 ppy (IQR 3.16-6.8) despite 98% compliance with LLITNs. There were only 6 cases of complicated malaria, all due to single convulsions. Malaria transmission was year-round, with two annual incidence peaks from Nov-Jan and Apr-Jul, corresponding to rainy seasons. Malaria incidence peaked at 6.5 ppy at 23 months of age before declining to 3.5 ppy at 48 months of age. After adjusting for age and season, the relative risk of malaria increased by 52% from 2008 to 2011 (RR 1.52, 95% CI 1.10-2.09). Asymptomatic parasitemia was uncommon (monthly prevalence <10%), and was rarely detectable prior to 24 months of age. Despite LLITNs and prompt treatment with ACTs, the incidence of malaria is very high in Tororo and appears to be rising. Additional malaria control interventions among young children living in high transmission settings are needed.

## RELATIONSHIP BETWEEN PARASITE BURDEN AND SEVERITY OF MALARIA ILLNESS IN AFRICAN CHILDREN

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Severe *Plasmodium falciparum* malaria is a major cause of pediatric mortality. The contribution of parasite burden to the pathogenesis of severe malaria has been controversial. We documented *P. falciparum* infection and disease in 882 Tanzanians followed from birth up to age 4 years. 102/882 children experienced severe malaria, but only 3 suffered more than 2 episodes. Risk of first severe episode was stable over several infections. Mean parasite levels were higher during severe disease, but overlapped considerably with those of children with mild malaria at the same age. 21/102 children experienced high density infection with no more than mild symptoms before having severe malaria. Severe malaria incidence rapidly decreased after infancy, while incidence of high density infection (>100,000 parasites/ $\mu$ l blood) increased. Mean parasite densities were similar during infections before versus after severe malaria episodes; 55/102 children experienced high density infections afterward with mild symptoms. Bed nets, maternal malaria, malaria season, and sickle trait modified severe malaria risk, and also modified parasite density during infections. Based on these data, resistance to severe malaria is not acquired after 1-2 mild infections. Although parasite burden on average is higher during severe malaria episodes, high parasite burden is often insufficient to cause severe malaria even in children who later prove to be susceptible. The diverging rates of severe disease and high density infection after infancy, and similar parasite burdens before and after severe malaria, indicate that naturally acquired resistance is not related to improved control of parasite density.

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### RISK OF READMISSION OR DEATH WITHIN SIX MONTHS AFTER INITIAL DISCHARGE AMONG UGANDAN CHILDREN WITH SEVERE MALARIAL ANEMIA AND CEREBRAL MALARIA

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Severe malarial anemia (SMA) is a leading cause of morbidity and mortality among young children in sub-Saharan Africa. Children with SMA appear to have an elevated risk for re-hospitalization and death during the first 6 months following discharge, but the risk of hospital readmission and death for children with cerebral malaria (CM) has not been assessed. We compared the risk of readmission to hospital or death within 6 months of admission in Ugandan children aged 18 mo - 12 y who were successfully treated for CM or SMA Mulago Hospital, Kampala, Uganda and then followed up for 6 months. Children with sickle cell disease were excluded from this analysis. Eligible children with CM (n=167) or SMA (n=144) were compared to asymptomatic community children (n=160). The primary endpoint, readmission or death, was infrequent in CC (5%), but more frequent in children with SMA (25.7%,  $P<0.0001$ ) or CM (16.8%,  $P=0.0007$ ). Readmission or death was higher in children with SMA than children with CM ( $P=0.07$ ). Assessing individual outcomes, frequency of readmission within 6 months of discharge was higher in children with SMA (21.5 %) or CM (16.2 %) as compared to CC (5%,  $P<0.0001$  for SMA,  $P=0.001$  for CM), but did not differ significantly between children with CM and children with SMA ( $P=0.22$ ). Most hospitalizations were for malaria, though on many hospitalizations no blood smear confirmation was obtained. Frequency of death was also higher in children with SMA (3.4%) as compared to CC (0%,  $P=0.02$ ) or children with CM (0.6%,  $P=0.07$ ). Most deaths were reported as due to a febrile illness. Children with SMA and CM have a higher risk of readmission within 6 months after discharge than community children, and children with SMA have a greater risk of death within 6 months of discharge than children with CM or community children. Further study is needed to assess causes of readmission or death in children with severe malaria.

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### IDENTIFICATION OF HOTSPOTS OF MALARIA TRANSMISSION IN WESTERN KENYAN HIGHLANDS; PAVING THE ROAD TO TARGETED AND EFFECTIVE INTERVENTION STRATEGIES

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Malaria risk is not uniformly distributed between districts, villages and even households from the same village. Some households are disproportionately exposed to malaria and incidence in these households may be more than 5-fold higher than the village mean. This micro-heterogeneity in the burden of malaria is largely explained by variation in exposure to infected mosquito bites and may complicate malaria control by resulting in large variations in the impact of interventions. Whilst mean levels of malaria transmission may decline in an area, strongholds of intense transmission may persist even during the seasons of low transmission intensity. In the highlands of western Kenya, malaria transmission is highly variable despite universal high coverage rates with insecticide treated nets (ITNs) and indoor residual spraying (IRS). In Rachuonyo South district, community surveys that were conducted in 2010 indicated parasite prevalence averaging 14.8% but ranging from 0% to 51.5% this effect seemed more pronounced in children aged 8-14 years where parasite prevalence was on average 25.8% but ranged from 0 to 71.4%. The true extent of heterogeneity in these areas remains unknown

but it is likely to have an impact on effective malaria control. In a recent cross-sectional survey, we attempted to identify micro-epidemiological patterns in malaria transmission in the Rachuonyo South district. We collected blood samples from approximately 3,200 households comprising 17600 individuals, in an area of 100km<sup>2</sup>. Every household was mapped with a GPS device. Hotspots of malaria transmission were identified by determining spatial patterns in the prevalence of *Plasmodium falciparum* parasites, determined by PCR analyses (23.0% positive, 95% CI 22.1-24.0%) and serological markers of malaria exposure based on the levels of AMA-1 (44.4% positive, 95% CI 43.6-45.2%) and MSP-119 (34.4% positive, 33.7-35.1%), antibodies. Serological markers of malaria exposure have the advantage of providing information on malaria exposure that is less susceptible to seasonal variations in the force of infection. 29.3% of the people (95% CI 28.7-30.0%) lives inside hotspots of more intense malaria transmission as detected by parasite prevalence and density of malaria specific antibodies ( $p<=0.05$ ). The identified 'hotspots' of malaria were then used for a targeted intervention strategy deployed in March 2012. The impact of this strategy is currently under evaluation.

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### THE SPATIAL AND TEMPORAL HETEROGENEITY OF ASYMPTOMATIC PLASMODIUM FALCIPARUM PARASITEMIA AMONG KENYAN SCHOOL CHILDREN

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As malaria transmission intensity declines and infection heterogeneity increases, identifying and treating "hotspots" is becoming increasingly important in the fight for elimination of malaria. Community based surveys are logistically complex and costly, but schools could be effective platforms for identifying such "hotspots" if school-based screening demonstrates clusters of infection. This study investigates the spatial and temporal patterns of asymptomatic *Plasmodium falciparum* infection in schoolchildren and the ecological and sociodemographic predictors of such patterns. A cohort of 2400 children from 51 primary schools were tracked at six time points, 2010-2012, as part of a trial investigating the impact of school-based malaria control on the south coast of Kenya. Demographic, socioeconomic and bednet use data were recorded for each child and households of children in 26 schools were mapped. Environmental covariate information was derived from high-resolution satellite data and spatially explicit Bayesian hierarchical modelling was used to examine clustering at school and household levels. Marked geographical heterogeneity of *P.falciparum* infection was observed, with persistently high transmission exhibited in a minority of schools. High rates of reinfection and the clustering of infection across small groups of households over time were observed, and indicate "hotspots" at the local level. The strong infection heterogeneity, persistent over time, points to a role for school-level screening in active detection of clusters in the wider community, and suggest a role for reactive screening and treatment of other members of households of positive cases identified in schools.

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### HOUSEHOLD MALARIA PARASITE BIOMASS AND SUBSEQUENT RISK OF SYMPTOMATIC MALARIA IN CHILDREN IN TANZANIA

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Hotspots of malaria transmission are likely to occur at all levels of transmission. One component that supports a hotspot is the presence of semi-immune asymptomatic carriers of malaria parasites that are clustered in households. We hypothesize that this asymptomatic pool of parasites is responsible for increased transmission of malaria. Thus household parasite biomass is a determinant of the risk of symptomatic malaria in children under the age of 5 years. The study was carried out in four villages in Mwanza region, Tanzania, an area with moderate transmission of *P. falciparum*. In a cross sectional survey, carried out in the dry season between August- October 2010. Blood samples to detect parasite biomass at household level were collected from all household members. Passive case detection of symptomatic malaria cases took place at health facilities located at the study site for one calendar year following the survey. Quantitative Polymerase Chain Reaction (qPCR) was used to determine biomass at the household level. Geospatial analysis was used to define households and clusters with high dry season parasite biomass and logistic regression modeling was used to determine the predictors of subsequent risk of malaria in children under 5 years of age. Children living in households with higher parasite biomass had three times the risk of having a subsequent attack of symptomatic malaria detected by passive surveillance compared to households with low parasite biomass. Clustering of parasite biomass was associated with the incidence of symptomatic malaria. These results support the hotspot theory of malaria and suggest that targeting asymptomatic carriage in households will reduce the incidence of malaria in young children.

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### SHRINKING THE MALARIA MAP IN MULEBA DISTRICT, NORTHWESTERN TANZANIA: MONITORING THE IMPACT OF MALARIA PREVENTION INTERVENTIONS SCALE-UP THROUGH HOSPITAL ADMISSIONS

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Muleba district (population of 450,000) in north-western Tanzania, an unstable *Plasmodium falciparum* transmission area, experienced severe malaria outbreaks in 2006/7. In 2007, indoor residual spraying (IRS) was introduced as an outbreak preemptive measure covering about 200,000 people. One IRS round per year was applied in the entire district since 2009. About 80,000 long-lasting insecticidal nets (LLINs) were distributed to under-fives in 2009. In 2011 another 170,000 LLINs were distributed to cover the remaining population. Malaria admissions can be considered a proxy indicator of the occurrence of severe malaria and, hence, intensity of malaria transmission. In 2006 a special surveillance system was established at five health facilities with in-patient services in the district. All patients with a laboratory confirmed diagnosis of malaria were recorded and entered into a database (age, gender, dwelling, disease outcome, date of admission). Between 2006 and 2011 a total of 45,512 cases were recorded and 32,839 (72%) were under-five years of age. The database allowed monitoring and mapping of severe malaria morbidity trends at different intervals and administrative units. Malaria admission rate

(number of admission per 1000 population) among under-fives declined from 145 to 20 per 1000 (86% reduction) between 2006 and 2010, with significant variation in decline among district locations. An increased malaria admission rate (70 per 1000) was then observed in 2011 with high variation among the different location from 0/1000 to 99 per 1000. Several factors contributed to this increase, including acute ACT stock-outs in health facilities, decreased susceptibility to the insecticide used for IRS, abnormal precipitation, and low net use. The experience in Muleba demonstrates the importance of surveillance to monitor disease trends after malaria intervention scale-up and to stratify malaria transmission risk to help detect and locate abnormal increases in transmission. Carefully-coordinated surveillance and response are required to address ongoing, low-level transmission hot spots as well as acute outbreaks once control of malaria is achieved.

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### SEASONAL MALARIA CHEMOPREVENTION AND COMMUNITY CASE MANAGEMENT FOR MALARIA IN SOUTHERN SENEGAL: A CLUSTER-RANDOMIZED TRIAL

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Although the overall incidence of malaria has recently declined in Senegal as in some other countries, this hides the fact that the burden of malaria remains very high in some parts of the country, such as Saraya district, where 70% of the community lives more than 15km from the nearest health post. Community case management for malaria is being introduced, volunteers (Distributeurs de soins à domicile or DSDOM) are trained to recognize the signs and symptoms of uncomplicated and severe malaria, to use Rapid Diagnostic Tests, and to treat malaria with artemisinin combination therapy. The DSDOM could also deliver Seasonal Malaria Chemoprevention (SMC) to children, SMC is known to be highly effective in preventing malaria illness but the relative advantage of adding SMC in villages which have access to prompt effective treatment from the village health worker has not been evaluated. In this trial, 24 villages were randomized to deliver SMC with community case management, or community case management alone. In SMC villages, the DSDOM gave all children under 10 years old preventive treatment with sulfadoxine-pyrimethamine plus amodiaquine each month from July to November 2011. Previously SMC has been delivered over three months so this study will also provide new evidence about the feasibility, tolerability and acceptability of delivery over a longer period. The DSDOM were trained to make blood films which were collected by the study team so that malaria cases could be confirmed by microscopy. The impact of SMC on drug resistance is being evaluated by analysis of used RDTs and from blood samples taken from a sample of children at the end of the transmission season. The added value of SMC will be discussed, and data on the impact of the intervention on malaria, anaemia and the safety profile of 5 doses of SMC will be presented.

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### RANDOMIZED CONTROLLED TRIAL OF LEVAMISOLE HYDROCHLORIDE AS ADJUNCTIVE THERAPY IN SEVERE FALCIPARUM MALARIA WITH HIGH PARASITEMIA

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Cytoadherence and sequestration of red blood cells containing the mature stages of *Plasmodium falciparum* are central to the pathogenesis of severe malaria. The oral antihelminth drug levamisole inhibits cytoadherence *in vitro* and reduces sequestration of late stage parasites in uncomplicated *falciparum* malaria treated with quinine. We did an open label randomised controlled trial to assess the benefit of levamisole as adjuvant treatment in adults patients with severe *falciparum* malaria treated with artesunate. A total of 56 adult patients with severe malaria and high parasitaemia admitted to a referral hospital in Bangladesh were randomised to receive a single dose of levamisole hydrochloride (150 mg) or no adjuvant to antimalarial treatment with intravenous artesunate. Main outcome measures were the kinetics of late stage peripheral blood parasitaemia, reversal of lactic acidosis and rectal capillary blood flow by orthogonal polarising spectroscopy. Circulating late stage parasites measured as the area under the parasite clearance curves for late trophozoite and schizont stage parasites was 2150 (0-28,025) parasites/ $\mu$ l\*h in patients treated with levamisole versus 5489 (192-25848) parasites/ $\mu$ l\*h in controls ( $p=0.254$ ). The 'sequestration ratios' at 6 and 12 hours for all parasite stages were no different between the groups. The time to normalisation of plasma lactate (<2 mmol/L) was 24 (12-30) hours with levamisole versus 28 (12-36) hours without ( $p=0.148$ ). There was no benefit of levamisole hydrochloride as adjuvant to intravenous artesunate in the treatment of adults with severe *falciparum* malaria. The potent killing of ring stage parasites by intravenous artesunate, preventing their further maturation and sequestration, might obscure the effects of levamisole in preventing sequestration.

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### EVALUATING THE IMPACT OF ENHANCED HEALTH FACILITY-BASED CARE FOR MALARIA AND FEBRILE ILLNESSES IN CHILDREN IN UGANDA: THE ACT PRIME STUDY

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Early diagnosis and prompt effective treatment reduces morbidity and mortality from malaria. However, inadequate health services limit appropriate fever case management in the public sector. We are conducting a cluster randomized trial in Tororo, Uganda to assess whether enhancing lower-level government health centers improves appropriateness of antimalarial treatment and patient satisfaction. Twenty health centers have been randomized; 10 to the intervention, and 10 to standard care. The intervention, which began in May 2011, includes training in health center management, fever case management, and

patient-centered services, and provision of rapid diagnostic tests (RDTs) for malaria and artemether-lumefantrine when stocks run low. We are conducting a series of evaluations interviewing caregivers of children under five years as they exit health centers. Information is gathered about the purpose of the visit, diagnostic testing for malaria, diagnosis given, and medications prescribed and received, and satisfaction with the visit to the health center. If the child has fever or history of fever, a RDT is performed. Study outcomes include (1) the proportion of children with suspected malaria who were inappropriately treated for malaria, based on the result of the research RDT, considering those children with a negative RDT who were given artemisinin-based combination therapy (ACT) plus those with a positive RDT who were not given an ACT, (2) the proportion of children with a positive RDT who were inappropriately treated with a non-ACT regimen, and (3) patient satisfaction with their experience. Two rounds of interviews have been carried out, in August 2011 and February 2012. In each survey, caregivers of 200 children were interviewed, including 10 from each facility. Full results from three rounds of interviews will be presented, providing much needed evidence of the effectiveness and sustainability of a complex health facility-based intervention on provider practices and patient management.

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### A NEW APPROACH FOR MALARIA CONTROL IN SCHOOLS: RESULTS OF A RANDOMIZED TRIAL OF INTERMITTENT PARASITE CLEARANCE

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Malaria control has traditionally focused on pregnant women and children under five years, in whom the risk of malaria-related mortality is greatest. Yet recent studies have shown that older school-age children could also benefit from malaria control, with potential gains for both health and education. Senegal recently introduced universal coverage of nets, with national roll-out of community-wide distributions starting in 2010. Whilst successful in achieving high levels of coverage amongst school-children in Kedougou, over 30% of children remained infected at the end of the malaria transmission season in December 2010. This calls for additional control measures in this age group. One potential supplementary strategy is intermittent parasite clearance in schools (in which a treatment dose is given irrespective of infection status) with the aim to improve educational performance by reducing malaria-related anaemia and improving cognitive function among schoolchildren. This approach is particularly suited to areas of seasonal transmission where a single annual treatment can be given at the end of the transmission season. To evaluate this approach, a randomized double-blind placebo-controlled trial was conducted amongst children already sleeping under insecticide-treated nets in Kedougou, Senegal. Children enrolled in six primary schools (7-14 years) were individually randomized to either receive IPC with sulphadoxine-pyrimethamine and amodioquine (SP+AQ), or placebo (n=865). Following informed parental consent and baseline parasitological survey, children received a single treatment dose in school at the end of November 2011. IPC treatment was administered over three days by the research team. The impact of the intervention on malaria parasitaemia, anaemia, and tests of sustained attention was evaluated through a transverse survey in February 2012. The advantages and disadvantages of this control approach will be discussed, and data on the impact of the intervention on malaria, anaemia and cognitive function at follow-up will be presented.



### ASSESSING THE EFFECT OF THE RECOMMENDED DIHYDROARTEMISININ-PIPERAQUINE DOSING REGIMEN ON THE RISK OF TREATMENT FAILURE IN PATIENTS DIAGNOSED WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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The fixed dose antimalarial dihydroartemisinin-piperaquine (DHA-PQ) is administered according to weight or age banding. To assess whether dosing strategies and total dose of piperaquine were associated with treatment efficacy, individual patient data (N=3573) were collated from 11 clinical efficacy studies of uncomplicated *P. falciparum* conducted between 2003 and 2010 with a follow up of 28 days or longer [Africa:2427, Asia:1146]. There were a total of 59 recrudescence and 336 new infections. The spread of PQ dosage was greatest in children <5 years (median=55.6 mg/kg, IQR: 47.5-64.0 mg/kg) compared to patients aged 5-15 (median=55.6 mg/kg, IQR: 51.2-61.9 mg/kg) and >15 years (median=51.1 mg/kg, IQR: 47.4-54.3 mg/kg). Children <5 years were at greatest risk of recrudescence (2.2%, 52/2371) compared to those aged 5-15 (0.9%, 4/457) and >15 (0.4%, 3/745). Patients failing treatment received a lower dose of PQ (median: 48.9mg/kg, IQR: 43.6-52.1) compared to those who were cured (median: 53.3mg/kg, IQR: 48.0-60.0). In the multivariate analysis an overall PQ dose < 48 mg/kg (AHR=1.7 [95% CI: 0.9-3.0],  $P=0.0720$ ) and an age <12 years (AHR: 4.7 [95% CI: 1.3-17.8],  $P=0.0220$ ) were independent risk factor for recrudescence. PQ dose <48 mg/kg was administered in 25.2% (201/2371) of children aged <5 years, 9.4% (43/457) in 5-15 age band and 26.9%(201/745) in those aged >15. In the children <15 years, the failure rate was 1.8 times greater in those who received a mg/kg dose below the 48 mg/kg threshold (3.17%, 20/631) compared to those who received >48 mg/kg (1.67%, 36/2150). The efficacy of DHA-PQ is vulnerable to under dosing particularly in young children. The lower mg/kg dose of PQ in patients who failed treatment and higher failure rates in children suggest that further dosing optimization to ensure adequate efficacy in young children may be warranted.

### IN VIVO EFFICACY OF SULFADOXINE-PYRIMETHAMINE FOR THE TREATMENT OF ASYMPTOMATIC PARASITEMIA IN PREGNANT WOMEN IN MALAWI

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Malaria in pregnancy is associated with severe maternal anemia, placental parasitemia, low birth weight, and increased perinatal mortality, especially among primigravidae. Sulfadoxine-pyrimethamine (SP) is recommended for intermittent preventive treatment in pregnancy (IPTp), but its effectiveness might be compromised by high prevalence of SP resistance. We assessed the *in vivo* efficacy of SP in asymptomatic parasitemic women as proxy for SP effectiveness in this vulnerable population. Pregnant women between 16 and 26 weeks gestation with asymptomatic parasitemia who presented for antenatal care at Machinga District Hospital in Malawi, were given SP and followed for 42 days. Survival analysis was conducted to determine drug efficacy. We included 245 pregnant women of whom 150 reached a valid study endpoint. The survival rate was 64.5% (95% confidence interval (CI) 58.3- 70.3%). Of those who experienced recurrent parasitemia, polymerase chain reaction (PCR) could be done on 78% to differentiate reinfection from recrudescence (13 could not be amplified and 6 were unavailable). Of those with PCR data, 75% had recrudescence.

Missing PCR results were assumed to follow a similar distribution with 75% due to recrudescence. The PCR corrected survival was 73.1% (95% CI 67.2- 78.3%). Recrudescence was more common among primi- than among multigravidae, with a recrudescence rate of 33.3% (95% CI 25.1- 42.4%) and 21.4% (95% CI 15.0- 29.0%), respectively (log rank test  $p$ -value 0.006). SP had low *in vivo* efficacy in asymptomatic parasitemic pregnant women, especially primigravidae. New efficacious antimalarials are needed to prevent malaria in pregnancy.

### ASSOCIATIONS BETWEEN INTERMITTENT PREVENTIVE THERAPY WITH SULFADOXINE-PYRIMETHAMINE AND PLACENTAL MALARIA IN AN AREA OF HIGH ANTIFOLATE RESISTANCE IN TORORO, UGANDA

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Intermittent preventive treatment with sulfadoxine-pyrimethamine during pregnancy (IPTp-SP) is widely recommended in sub-Saharan Africa to reduce the risk of placental malaria (PM) and the adverse birth outcomes associated with this disease. However, there are reports that the efficacy of IPTp-SP is waning, especially in parts of East Africa where antimalarial resistance to antifolate drugs has become widespread. We conducted a cross-sectional study of 565 HIV uninfected women giving birth at Tororo District Hospital, an area of Eastern Uganda with high transmission intensity. The primary objective of the study was to measure the association between reported use of SP during pregnancy (from antenatal cards) and the risk of PM defined as any presence of asexual parasites or hemozoin pigment using placental histopathology. The proportion of women who reported taking 0, 1, 2 and 3 doses of SP during pregnancy was 6%, 36%, 57% and 2% women, respectively. The prevalence of placental malaria was 74% among women who reported taking 0-1 doses of SP and 60% among women who reported taking 2-3 doses of SP. Using multivariate logistic regression, women who reported taking 2-3 doses of SP had a lower odds of PM (OR=0.55, 95% CI 0.32- 0.96,  $p=0.04$ ) compared to those who reported taking 0-1 dose of SP. Compared to multigravida women, those who were secundagravida (OR=3.11,  $p=0.001$ ) or primagravida (OR=6.13,  $p<0.001$ ) had a higher odds of PM. Reported use of insecticide treated bed nets was associated with a lower odds of PM (OR=0.57,  $p=0.05$ ) and women from households with a wealth index below the median had a higher odds of PM (OR=3.30,  $p<0.001$ ). There was no association between the woman's education level and the odds of PM. The prevalence of placental malaria by histopathology was very high in an area of Uganda with high transmission intensity. The reported use of 2-3 doses of SP was associated with modest protection against PM despite widespread antifolate resistance which has been previously reported from this area.

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**MATING-INDUCED GENE EXPRESSION CHANGES IN THE SPERMATHECA OF ANOPHELES GAMBIAE**

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The high reproductive success of the malarial vector mosquito *Anopheles gambiae* is ensured by a single mating event; females store sperm from the male in a sperm storage organ called the spermatheca, using them to fertilize eggs which they develop after each blood meal. Sperm transferred during mating must be stored reliably to protect sperm quality from stresses encountered in the female such as free radicals produced during blood meal digestion. As *Anopheles* females mate only once and sperm are not replenished, mechanisms ensuring sperm viability must be functional for the lifetime of the female. Targeting sperm storage mechanisms in the female therefore would provide an alternative control strategy for reducing vector numbers and hence contribute to malaria control. As a step towards understanding the molecular mechanisms of sperm storage in female mosquitoes, we compared gene expression profiles of spermathecae 24 hours post-mating to age-matched virgins by genome-wide microarrays. We detected over 200 genes significantly up- or down-regulated in the sperm storage organs by mating. Mating-induced transcriptional changes were validated by qRT-PCR on a subset of genes in independent samples. Functional analysis of gene ontology terms showed up-regulated genes were involved in ion and sugar transport, suggesting a requirement for a specific nutrient environment within the spermatheca. Both up- and down-regulated genes contained annotations for diverse metabolic processes, in particular, protease metabolism. In addition, up-regulated genes also included components of several signalling pathways, suggesting signalling between the spermathecal contents and the surrounding cells. To investigate these signals further, we analysed the lower reproductive tracts of females mated to males lacking functional testes (and producing no sperm) and found that these expression changes did not require sperm. We hypothesise that additional small molecules from the male, either produced by the male accessory glands, or processed in the spermatheca itself, could trigger gene expression changes to preserve sperm quality. Functional analysis of candidate genes using RNA interference is highlighting mechanisms required for long-term sperm viability. These candidates could be targeted in the field to reduce the fertility of natural mosquito populations.

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**INFLUENCES OF MALE SEMINAL FLUID PROTEINS IN THE ABSENCE OF SPERM ON FEMALE BLOOD FEEDING AND SEXUAL RECEPTIVITY FOR THE DENGUE VECTORS, Aedes Aegypti AND Ae. Albopictus**

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As part of a larger study on the function of male seminal fluid proteins (SFP) in *Aedes* mosquitoes, we investigated the effect of SFPs in combination with and without sperm on female blood feeding behavior and life history traits for *Ae. aegypti* (human blood meal volume ingested, digestion rate, feeding frequency, egg production and survival). In addition, we determined the dose-dependent effect of SFPs in the absence of sperm on female mating behavior in both *Ae. aegypti* and *Ae. albopictus*. Injected SFP homogenates stimulated egg production and feeding frequency to the level of naturally mated females. SFPs did not affect blood meal volume ingested, digestion rate, or survival. In addition, female *Ae. aegypti* and *Ae. albopictus* demonstrated high sensitivity to SFPs from their own species, with the majority of females becoming

refractory to mating at doses  $\geq 0.03$  male-equivalents. This effect persisted up to thirty-four days post-injection. These results will aid future work to characterize individual SFPs involved in post-mating effects for these two important mosquito vectors.

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**HETEROCHROMATIN PATTERNS IN MITOTIC CHROMOSOMES OF SPECIES FROM THE ANOPHELES GAMBIAE COMPLEX**

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The *Anopheles gambiae* complex is a cluster of seven morphologically indistinguishable species. These species have distinct ecological and physiological adaptations apart from difference in vector capacity. *An. gambiae*, the major African malaria vector, is undergoing incipient speciation into two molecular forms, M and S. These two forms exhibit distinct differences in larval habitat, ecology, oviposition and behavior in the presence of predators. Heterochromatin is a gene-poor repeat-rich part of the eukaryotic genome. It makes up about one fourth of the *An. gambiae* genome. Heterochromatin plays a vital role in several biological functions, including chromosome function and gene regulation. It is also associated with rapid evolution and speciation. Here we studied heterochromatin patterns in the *An. gambiae* complex to evaluate if possible differences in heterochromatin may provide a key to better understanding of adaptation and speciation. Mitotic preparations obtained from imaginal discs of larvae were stained with two fluorescent dyes, DAPI and YOYO-1. We found differences in the number and size of the X chromosome heterochromatin bands throughout the *An. gambiae* complex. The laboratory strains of species showed a gradual increase in the amount of heterochromatin from *An. arabiensis* to *An. quadriannulatus* to *An. gambiae* and to *An. merus*. Laboratory strains tested for the M form (Akron and SUA2La) had a proximal heterochromatin band when stained with DAPI. In contrast, the laboratory strains tested for the S form (Pimperena, Zanu and Kisumu) exhibited both proximal and distal heterochromatin bands. We conclude that sibling species of the *An. gambiae* complex have fixed differences in the pattern and amount of the X chromosome heterochromatin. Future work would determine if natural populations of these species exhibit fixed heterochromatin differences and could lead to identification of possible causative links between heterochromatin variations and ecological adaptations of malaria vectors.

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**PHYSICAL MAP OF Aedes Aegypti GENOME**

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Mosquitoes are vectors of numerous deadly human diseases. To facilitate the development of new strategies for vector control, the genomes of the three most dangerous species of mosquitoes had been sequenced in the last decade. Among these three species, the vector of dengue and yellow fever viruses, *Aedes aegypti*, has the largest genome with 1,310 Mb, which is hard to assemble and map to chromosomes. Here, we report the first band-based chromosomal map of the donovani genome. Idiograms for the mitotic chromosomes of *Ae. aegypti* were developed. Three chromosomes were subdivided into 23 regions and 94 subdivisions. Instead of previously used cell lines, which usually accumulate chromosomal rearrangements, our method utilized chromosomes from a live mosquito. Based on results of fluorescent in situ hybridization (FISH), a total of 500 BAC clones from the largest genomic supercontigs was assigned to the specific band onto idiograms. The BAC clone locations within the supercontigs were predicted by fingerprint analysis. BAC clones were directly labeled with Cy3 and Cy5 fluorescent dyes by nick-

translation. Unspecific hybridization was prevented by adding unlabeled repetitive DNA fractions to the probe. From all BAC clones 106 were carrying previously mapped major genetic markers. These BAC clones were additionally ordered within each band by multicolor FISH because of the importance to link their genomic locations to the genetic locations of quantitative trait loci (QTL) related to pathogen transmission. The current study placed ~50% of the *Ae. aegypti* genome to precise chromosomal positions and also combined cytogenetic, genetic and genome maps into one integrated physical map (iMap) of the yellow fever mosquito. Further application of this map will enhance the quality of the current genome assembly of *Ae. aegypti* and also will help to find the genomic locations of QTL that might be important targets for developing advanced genome-based strategies for vector/disease control.

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### SEARCHING FOR THE CULICINE MALE DETERMINING LOCUS

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*Culex* and *Aedes* mosquitoes that vector deadly arboviruses such as West Nile virus, yellow fever, and dengue do not have heteromorphic sex chromosomes. Rather, sex is thought to be determined by a dominant autosomal "male determining locus" (MDL). The exact location, sequence, and gene product of the MDL remain unknown. We show that *Culex pipiens pallens* is the result of asymmetrical hybridization between putative *Cx. pipiens* females and *Cx. quinquefasciatus* males, two species that although closely related have unique genetic signatures. Significantly, as a result of non-recombination around critical sex loci, *Cx. p. pallens* males exhibit a DNA fragment identical to that of *Cx. quinquefasciatus*, and thus mosquito gender can be identified at any life stage. We have optimized SNPs focused on the region of the genome where we have evidence the MDL is located. We will discuss the impact of hybridization in mosquito evolution and control.

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### MITOCHONDRIAL GENOME SEQUENCES REVEAL DEEP DIVERGENCES WITHIN THE ANOPHELES PUNCTULATUS GROUP

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Members of the *Anopheles punctulatus* group (AP group) are the primary vectors of human malaria and lymphatic filariasis in Papua New Guinea. Given their public health importance it is critical that we understand the species diversity and evolutionary history of *Anopheles*, for example, to determine why only certain mosquito species can transmit malaria and other human diseases. Here, we present the complete DNA sequences of 13 mitochondrial genomes from 7 distinct species: 5 from AP sibling species and 2 from the *An. dirus* complex in Southeast Asia. We assembled four sequences directly from whole genome sequencing data, while the remaining 9 mitochondrial genomes were sequenced simultaneously on one lane of an Illumina HiSeq 2000 instrument (after individual adapter-based barcoding). Our phylogenetic reconstruction suggests that the ancestor of the AP group arrived in Papua New Guinea 20 to 40 million years ago. Our results also reveal a deep divergence between *An. punctulatus* s.s and the *An. farauti* clade occurring 26 to 33 million years ago. This deep divergence within the AP group is interesting, as humans did not arrive in Papua New Guinea until 50 thousand years ago. We hypothesize that many malaria-related traits, such as human blood preference or the ability to carry human parasites, occurred

independently in each *An. punctulatus* sibling species, which provides an opportunity to map these traits using comparative genomic methods. This deep divergence among AP mosquitoes also suggests that gene flow between species is limited and, therefore, that insecticide resistance is unlikely to spread from one species to another but instead would have to occur independently in each species.

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### COMPARATIVE PHYLOGEOGRAPHY OF MALARIA MOSQUITOES FROM THE SOUTHWEST PACIFIC: POPULATION STRUCTURE, MITOCHONDRIAL INTROGRESSION AND THE REPEATED EVOLUTION OF NON-HUMAN BITING BEHAVIOR

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Using extensive geographical sampling, we examined and compared the phylogenetic relationships, phylogeography, and population structure of malaria vectors *Anopheles farauti* and *An. hinesorum* as well as the non-human biting *Anopheles irenicus* throughout their ranges in the southwest Pacific using mitochondrial (mtDNA COI) and nuclear (ribosomal protein S9 and ribosomal DNA ITS2) loci. Phylogenetic analyses suggest that non-human biting behaviour has evolved repeatedly (in populations of *An. hinesorum* and in *An. irenicus*), coincident with independent colonizations of the Solomon Islands. Maximum likelihood and Bayesian phylogenetic analyses of nuclear loci also showed that the three species are monophyletic. However, putative introgression of *An. hinesorum* mtDNA onto a nuclear background of *An. farauti* was evident in populations from Queensland, Torres Strait and southern New Guinea. Haplotype networks and pair-wise  $F_{ST}$  values show that there is significant genetic structure within New Guinea and Australia in both *An. farauti* and *An. hinesorum*, consistent with a long-term history of low gene flow among populations. Since some of the species under investigation (as well as other closely related anopheline mosquitoes) transmit malaria in the region so this is a medically important finding with regards to potentially understanding the mechanisms behind human feeding behaviour.

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### THE EVOLUTION OF VIRULENCE OF WEST NILE VIRUS IN A MOSQUITO VECTOR: IMPLICATIONS FOR ARBOVIRUS ADAPTATION AND EVOLUTION

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Alterations to life-history traits resulting from infection of mosquito vectors with arthropod-borne viruses (arboviruses) could have significant effects on both vectorial capacity and the potential for arbovirus evolution and adaptation. Although arbovirus-vector interactions are generally characterized as benign, virulence has at times been noted. Previous studies with West Nile virus (WNV) demonstrated decreased fecundity in *Culex tarsalis* mosquitoes associated with WNV infection, yet no cost of infection in *Cx. pipiens* mosquitoes in terms of survival, fecundity, or blood-feeding behaviour. In order to evaluate the potential for trade-offs between viral and vector fitness, studies assessing life history traits in *Cx. pipiens* were repeated with a *Cx. pipiens* -adapted WNV (WNV MP20). WNV MP20 was obtained by 20 sequential passages in *Cx. pipiens* and previously shown to have acquired both increased replicative ability and infectivity in *Cx. pipiens*. Results of current studies demonstrate that *Cx. pipiens* females fed on a WNV MP20 containing bloodmeal display significantly decreased survival relative to both unexposed or wildtype (WT) WNV-exposed mosquitoes. Specifically, WNV MP20-exposed females survived on average 16.9 days post-feeding, while mean survival of WT WNV-exposed and unexposed mosquitoes was 21.9 and 23.8 days,

respectively. In addition, both bloodfeeding behaviour and fecundity were altered in WNV MP20 exposed *Cx. pipiens* such that early feeding and fecundity were maximized at a later cost. When laboratory-evaluated differences in the probability of survival, bloodfeeding rates, and vector competence are used to compare vectorial capacities of *Cx. pipiens* infected with WT or MP20 WNV, results demonstrate that, despite increased viral fitness, vectorial capacity is in fact lower for the more adapted strain, demonstrating the inability of this strain to outcompete WT WNV on the population level. Although trade-offs between pathogen fitness and transmission have been identified in many other host-pathogen systems, these studies demonstrate for the first time that arbovirus evolution and adaptation may be constrained by the coupling of viral fitness and virulence in the vector.

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### WEST NILE VIRUS EVOLVES TOWARDS INCREASED AVIAN FITNESS IN CALIFORNIA

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The COAV997 strain of West Nile virus (WNV) isolated from Imperial County in July 2003 was the first detection of WNV in California. WNV subsequently spread throughout the state and has become endemic. To investigate the spatiotemporal phenotypic evolution of WNV in California, the fitness of sixteen WNV isolates from four ecologically different areas was evaluated competitively using an *in vivo* avian model. An infectious clone derived virus of COAV997 was genetically marked with five nucleotide mutations (COAV997mut) and competed against isolates made from mosquitoes collected along a S-N transect from Coachella Valley to Sacramento from late 2007 and from early, mid and late 2008. Isolates were mixed 1:1 with the COAV997mut and inoculated into House Finches. The outcome of competitive replication was determined by quantifying the number of RNA copies in the serum of viremic birds by specific qRT-PCR. Results indicated that isolates from Coachella Valley, an area geographically close to the origin of COAV997, have not changed markedly compared to strains from northern study sites that have undergone clear increases in replicative fitness. No coincidental gain in virulence (mortality) was observed with increased fitness, with the exception of a spring isolate collected prior to the 2008 Los Angeles outbreak. In conclusion, the phenotype of most California WNV isolates has evolved towards increased replicative fitness compared to the 2003 invading strain, but the extent of selective pressure may have varied due to geographical or perhaps vector competence differences.

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### EFFECT OF AVIAN COMMUNITY COMPOSITION AND MOSQUITO FEEDING PREFERENCE ON WEST NILE VIRUS (WNV) TRANSMISSION IN URBAN ENVIRONMENTS

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Heterogeneity in both mosquito biting behavior and avian reservoir competence are two important factors in the enzootic transmission cycle of West Nile Virus (WNV). The feeding preference for certain avian species among mosquitoes belonging to *Culex pipiens* complex is well documented. In addition, results from laboratory and field studies suggest

that certain avian species act as more competent reservoirs than others in terms of amplification and transmission of WNV. Consequently, avian community composition is a key factor in the persistence and spread of WNV in urban area. We developed a fine scale Individual Based Model (IBM) model for enzootic WNV transmission which simultaneously explores the roles of mosquito feeding preference in an avian community as well as variation in reservoir competence within that community. This modeling approach allowed us to investigate the effects of heterogeneity among individual birds on the transmission dynamics of WNV and the role of individual birds that act as super-spreaders. Interactions among vector and host were described using a network in which links between the nodes (mosquitoes and birds) represented feeding events. We defined three avian classes based on preferential feeding behavior of *Culex pipiens*: preferred, semi-preferred, and non-preferred hosts. We also defined two avian classes based on their reservoir competence: highly competent and semi-competent hosts. The heterogeneity attributed to each set of classes was set using results obtained by 1) blood meal analysis performed on engorged *Culex* mosquitoes and 2) WNV serology performed on wild passerines that were sampled in Atlanta, Georgia, United States. Different scenarios were also modeled using data extrapolated from studies performed in other urban areas in the United States. Both the basic reproductive number (R0) and the daily effective reproductive number (Rd) were calculated to determine the probability of having a WNV outbreak in the modeled avian community and to estimate the effect of avian community composition on the spread of disease (accounting for the population's reduced susceptibility during the course of an epidemic), respectively. Results suggest that avian community composition has a strong impact on the spread of WNV in the urban environment and help better understand the role of super spreader individuals in the transmission dynamics of WNV.

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### SHIFT IN DYNAMICS IN EASTERN EQUINE ENCEPHALITIS VIRUS ACTIVITY IN CENTRAL NEW YORK

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*Eastern equine encephalitis virus* (EEEV; *Togaviridae:Alphavirus*) is a highly pathogenic mosquito-borne virus that produces severe or fatal encephalitis in 30-50% of infected humans and horses. Recently, EEEV has undergone a regime shift in dynamics in the northeastern US and Canada. For three decades (1970-2000) periodic activity was detected in central NY, specifically in counties surrounding Oneida Lake, with 1-2 years of activity in mosquitoes and other hosts, followed by 1-6 years with no detectable activity. However, since 2003, there have been 9 consecutive years with active EEEV transmission, and a significantly higher prevalence of infection than in previous outbreaks ( $p = 0.009$ ). This string of 9 consecutive years is a highly unlikely event to occur by chance ( $p = 0.007$  with 15 of 28 years with EEEV presence). The current epizootic coincides with the arrival of a novel genotype of EEEV, the invasion of WNV to the region, and occurs within the backdrop of long term trends in temperature and declines in key songbird populations. The first human case in 26 years occurred in Oswego County in 2009 in a 70yo male, then again in 2010 in a 77yo male, and in 2011 in a 4yo child. EEEV-infected neurologic deer were found in 2008 and 2009. Serosurveys of hunter killed deer  $\leq 2$ yo also have proven useful in determining the range of EEEV activity with 9 EEEV positive deer out of 179 tested from central NY 2007-2009. In 2011, 46 *Culiseta melanura* pools were found positive (MIR 4.2); the previous 3 year average was 43 (MIR 4.1). Similarly, in 2011, 11 horses died from EEEV, while the previous 3 year average was 7 equines. In addition, two neurologic dogs died in 2011. Phylogenetic analysis of isolates indicates the virus is periodically introduced, most likely by migratory birds, but also overwinters in the central NY focus. However, we identified a genetic change in the virus in 2007, the year preceding the first human cases

seen since 1983. In summary, although the level of EEEV activity does not appear to be increasing significantly in the enzootic focus in central NY, it appears to be extending its range and intensity outside this historic focus.

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### CHOLUL VIRUS IS A NOVEL ORTHOBUNYAVIRUS REASSORTANT CREATED BETWEEN CACHE VALLEY AND POTOSI VIRUSES AND A CAUSE OF INFECTION OF HUMANS AND LIVESTOCK IN THE YUCATAN PENINSULA OF MEXICO

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We have discovered a novel orthobunyavirus reassortant in the Yucatan Peninsula of Mexico. This virus, tentatively been named Cholul virus (CHLV), acquired its small RNA segment from Cache Valley virus (CVV) and its medium and large RNA segments from Potosi virus. To determine the seroprevalence of CHLV and selected other orthobunyaviruses in livestock in the Yucatan Peninsula, a serosurvey was performed using sera from 255 domestic animals (182 horses, 31 sheep, 37 chickens, 5 turkeys). Sera were initially screened at a single dilution (1:20) by plaque reduction neutralization test (PRNT) using 6 orthobunyaviruses: CHLV, CVV, South River virus (SORV), Kairi virus (KRIV), Maguari virus (MAGV) and Wyeomyia virus (WYOV). If neutralizing antibodies were detected, the serum was further diluted and subsequent PRNTs were performed to determine the end-point titer. Of the 182 horses, 63 (34.6%) were seropositive for CHLV, 49 (26.9%) were seropositive for CVV, 1 (0.5%) was seropositive for SORV, 56 (30.8%) had antibodies to an undetermined orthobunyavirus and 13 (7.1%) were negative. Of the 31 sheep, 8 (25.8%) were seropositive for CHLV, 3 (9.7%) were seropositive for CVV, 4 (12.9%) were seropositive for SORV, 14 (45.2%) had antibodies to an undetermined orthobunyavirus and 2 (6.5%) were negative. Four (11%) chickens had antibodies to an undetermined orthobunyavirus and 1 (20%) turkey was seropositive for CHLV. To determine whether orthobunyaviruses are also responsible for human infections in the Yucatan Peninsula, sera from 823 febrile patients that reside in this region were examined at a 1:20 dilution by PRNT using CVV. A total of 146 (18%) individuals had antibodies that neutralized CVV. Fifty sera were further analyzed at multiple dilutions by PRNT using CHLV, CVV, SORV, KRIV, MAGV and WYOV. Six individuals were seropositive for CVV, 5 were seropositive for CHLV, 1 was seropositive for SORV and 38 had antibodies to an undetermined orthobunyavirus. In summary, we demonstrate that orthobunyaviruses are a common cause of infection of humans and livestock in the Yucatan Peninsula.

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### ARBOVIRUS SURVEILLANCE AND VIRUS ISOLATIONS FROM BATS IN UGANDA, KENYA, AND THE DEMOCRATIC REPUBLIC OF THE CONGO

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Zoonotic and vector-borne pathogens have comprised a significant component of emerging human diseases in recent decades. Arboviruses including Rift Valley fever (RVFV), Yellow fever (YFV), West Nile (WNV), Chikungunya (CHIKV) and Zika viruses have been isolated or detected serologically from various East African bat species, however the role of bats in arbovirus transmission cycles is poorly understood. The aim of this study was to investigate the exposure history of bats in East Africa to arboviruses as well as attempt virus isolation from bat tissues. Blood, liver, and spleen samples were collected from 281 bats from Uganda, 449 bats from Kenya, and 239 bats from the Democratic Republic of the Congo during 2011-2012. Liver and/or spleen samples from each bat were mechanically homogenized in tissue culture media and virus isolation was performed on Vero cells. Virus isolates were identified by either RT-PCR using virus group-specific primers, or by immunofluorescence assay. When available, serum samples were tested for neutralizing antibodies against WNV, YFV, Dengue 2 (DENV-2) virus, CHIKV, O'nyong-nyong virus, Babanki virus and RVFV by plaque reduction neutralization test. Virus isolates include Entebbe bat virus (*Flaviviridae: Flavivirus*) from *Chaerephon pumila* from Uganda, and as yet unidentified viruses from *Hipposideros commersoni* and *Triaenops persicus* in Kenya. *Epomophorus labiatus* fruit bats from Uganda demonstrated neutralizing antibodies against Babanki virus (1/33, 3%), RVFV (3/33, 9%), and flaviviruses (2/33, 6%). Flavivirus neutralizing antibodies were also found in *C. pumila* and *Mops condylura* from Uganda. Serological and virological evidence suggest that multiple species of fruit and insectivorous bats from East Africa are exposed to and are incidental or potential amplification hosts for arboviruses.

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### ASSOCIATION OF SANDFLY FEVER SICILIAN VIRUS WITH AN OUTBREAK OF ACUTE FEBRILE ILLNESS IN ETHIOPIA

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Sandfly Sicilian fever virus (SFSV) is a phlebovirus (family Bunyaviridae) transmitted by bites from sandflies. It was previously detected in the Mediterranean region with peaks reported in the summer in endemic areas. Clinical signs and symptoms range from a short, self-limited febrile syndrome to encephalitis and hemorrhagic fever. This study investigated the occurrence of an extensive cluster of cases of acute febrile illness (AFI) in the Afar region, Ethiopia. The Ethiopian Health & Nutrition Research

Institute (EHNR) requested KEMRI/CDC assistance with testing samples for Dengue fever as a recognized outbreak of dengue was ongoing in a neighboring area of Somalia at the time. Twenty-nine serum samples were screened by real time PCR for Dengue virus and selected viral hemorrhagic fevers. Subsequently, total nucleic acid extracts from these specimens were sent to Washington University in St. Louis for pathogen discovery using high throughput sequencing using the Roche/454 Titanium platform. Onset of symptoms was on September 5th, 2011; with case-patients reporting to the health facility starting three days later. A total of 12,816 cases were detected; of these, 9107 (71%) were male. The disease was described as self-limiting with symptoms resolving within 3 days of onset. All 29 serum samples tested negative for Dengue fever, Yellow Fever, Rift Valley Fever, Ebola-Zaire, Chikungunya Fever, Marburg and Crimean-Congo Hemorrhagic Fever Viruses. Computational analysis of the generated sequences identified 17 (59%) samples positive for SFSV, 1 for hepatitis B, and 2 positive for hepatitis GBV-C. Of the cases, 53% were female; the mean age of all patients was 28 years (range, 9 - 55 years, and median 25 years). All SFSV patients reported fever with periorbital and frontal headache. Other symptoms reported included, back pain (94%), loss of appetite (71%), joint pains (65%), vomiting/nausea (24%) and cough (18%) with no sore throat. This study indicates the occurrence of Sandfly Sicilian fever virus in the Afar region of Ethiopia. The resolution of symptoms after 3 days is similar to previous reports of this disease. This is the first study associating SFSV with an outbreak of AFI in Ethiopia. Detection of SFSV in these specimens by high throughput sequencing underscores the power of unbiased metagenomic strategies for pathogen detection. Additional studies are needed to determine the prevalence of Sandfly fever in this geographic area.

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### DEVELOPING AN ANTIMICROBIAL RESISTANCE STRATEGY FOR ENTERIC SURVEILLANCE ACTIVITIES WITHIN A NETWORK A GLOBAL PARTNERS

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Diarrheal illness poses a significant risk to deployed military personnel and can result in compromised operational readiness and effectiveness through lost work days and increased healthcare utilization. Through a network of global partners, the Armed Forces Health Surveillance Center's Division of Global Emerging Infections System (AFHSC-GEIS) conducts enteric infection surveillance in support of force health protection and global public health. Guidance for enteric surveillance priorities are provided by a steering committee consisting of experts and stakeholders within the military system. AFHSC-GEIS partners in Cambodia, Egypt, Kenya, Peru, Thailand and the US have focused enteric surveillance efforts in populations ranging from adult travelers and deployed US military to host country military and pediatric populations to gain a better understanding of diarrheal illness etiologies. With the growing concern of antimicrobial resistance in bacterial organisms, the AFHSC-GEIS Enteric Surveillance Steering Committee is developing an Antimicrobial Resistance Strategy (ARS). This strategy responds to the need for data to guide treatment of traveler's diarrhea, detect emerging antimicrobial resistance of global public health interest, and develop a multi-site platform to study molecular mechanisms of resistance. Given that treatment for diarrhea in a particular region may differ, the strategy identifies a matrix of 'bug-drug' combinations that all network partners should be using for antimicrobial susceptibility testing and lays out an approach for sampling and additional enteric antimicrobial resistance research priorities. This presentation summarizes the antimicrobial resistance priorities outlined in the ARS and the approach used by the partner network to generate descriptive data on spatial and temporal trends for antimicrobial drug susceptibility of important enteric bacterial pathogens.

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### PILOTING INTEGRATED COMMUNITY CASE MANAGEMENT OF MALARIA, PNEUMONIA AND DIARRHEA IN PRIVATE SECTOR DRUG SHOPS IN UGANDA

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Integrated Community Case Management (iCCM) of malaria, pneumonia and diarrhoea is typically implemented by lay community volunteers. However, the majority of febrile children in Uganda first seek care from the private sector, particularly at drug shops. The objective is to assess the feasibility and effects on quality of care of introducing diagnostics (malaria Rapid Diagnostic Tests and Respiratory Timers) and promoting pediatric-dosage pre-packed drugs for acute febrile illnesses (malaria and pneumonia) and diarrhea in private sector drug shops, in order to contribute to rational use of drugs, child survival and inform the introduction of private sector subsidy schemes. This is a proof of concept study with pre-post measurement using intervention and control districts. Subsidized drugs - Artemisinin Combination Therapy (ACTs), Amoxicillin tablets and Oral Rehydration Salts with Zinc (ORS/Zinc) - as well as diagnostics - malaria RDTs and Respiratory Timers - were introduced into all registered drug shops (n=44) in the intervention district. The control district continued to provide ACTs through the drug shops as was previously done. Household surveys, exit interviews at drug shops, in-depth interviews with drug sellers and focus group discussions with child-caretakers were conducted both at baseline in May 2011 and end line, in May 2012. At baseline, of children sick within the last 2 weeks, 496 (53.1%) first sought treatment in the private sector vs. 154 (16.5%) in a government health facility. Only 15 (10.3%) febrile children treated at drug shops received appropriate treatment for malaria. Of children with both cough and fast breathing, 5 (15.6%) received amoxicillin although none for 5-7 days. Of children with diarrhea, 8 (14.3%) received oral rehydration salts and none received zinc tablets. Supervision results indicate high utilization, that parents bring their sick children to drug shops for diagnostic testing, and high satisfaction among parents as well as drug shop staff. Results of the end line will be presented.

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### IMPACT OF PERFORMANCE-BASED FINANCING ON PRIMARY HEALTH CARE SERVICES IN HAITI

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To strengthen Haiti's primary health care (PHC), the country piloted performance-based financing (PBF) in 1999 and subsequently expanded the approach to most non-government organizations. Under PBF, these organizations receive incentives up to 10% of base (services) funding for key PHC coverage achievements. This study evaluates 1) the costs of PBF implementation and 2) the impact of PBF on PHC's service delivery in Haiti. We obtained quantities of key services from four departments for 217 health centers (15 with PBF and 202 without) for 2008 through 2010, and computed quarterly growth rates. We analyzed the results using a difference-in-differences approach in multiple regression analyses by examining the differences in the trend between incentivized and non-incentivized services between PBF and non-PBF facilities to partial out potential confounding factors. For interpretation, we also interviewed staff in four facilities. Whereas technical assistance (TA) added 39% to base costs of PHC, incentive payments, on average, added only 6%. TA alone increased the quantities of PHC services over three years by 35% (2.7%/quarter). However, TA plus incentives increased these amounts by 87%

over three years (5.7%/quarter) compared to facilities with neither input. The growth in volumes of services with incentives and TA was 52% higher than the increase in facilities with support alone. Thus, incentives more than doubled the growth of services, a significant improvement ( $p < 0.05$ ). Improvements related to use of PBF were particularly associated with the utilization of maternal child services. Interviews indicated beneficial impacts on quantity and quality of services through careful monitoring and found no adverse impacts. Incentives proved to be a relatively inexpensive, well accepted, and very effective complement to TA, suggesting that a small amount of money, strategically used, can substantially improve PHC. Haiti's experience indicates that incentives remain an effective tool to strengthen PHC after more than a decade of use.

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### WHO PAYS FOR MALARIA TREATMENT IN GHANA IN THE ERA OF HEALTH INSURANCE POLICY?

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Health insurance was instituted in 2005 as national policy by the government of Ghana to replace the cash and carry system of health care payment. This major financing reform in Ghana is a pro-poor intervention aimed at meeting basic health care needs of Ghanaians, with exemptions for vulnerable groups like children under five, pregnant women, and the aged. In recent years the out-of-pocket payments in national health insurance accredited health delivery facilities is rising. The paper investigates payment mechanisms households seeking treatment for malaria in Ghana use. It also assesses the socio-economic differentials among those using health insurance as a cushion for health care costs. The study is a cross sectional cost-of-illness study under the INDEPTH Network Effectiveness Safety Studies which employed quantitative data from the Dodowa Health and Demographic Surveillance System (HDSS) from October 2009 to December 2011. A household member who had been treated of fever within the last two weeks was interviewed about their expenditure on the treatment and the mechanism used to pay for the treatment. A total of 540 household members who received malaria treatment within the past two weeks were interviewed. Over 76% of household members paid out-of-pocket for treatment they received whereas 22% used health insurance and the remaining paid through an employer. An average of \$33 (€50.5) was borrowed by some patients to meet the health care cost. A bivariate analysis indicated that the poorest households are 90% more likely to pay out-of-pocket than the least poor (67%) for seeking malaria treatment. The analysis also showed that only 5% of the poorest patients are likely to use health insurance while the least poor are likely to use 42% of time, their health insurance to pay for treatment. Out-of-pocket payments for health care are still significant component of health care costs in Ghana despite the fact that the national health insurance is in operation. The poorest patients continue to suffer the burden of malaria treatment expenses and borrow to pay out-of-pocket for care.

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### OPTIMIZING CONTINUITY OF TREATMENT DURING UNREST IN KENYA, UGANDA, AND IVORY COAST

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This study explores the impact of emergencies caused by political unrest and violence on HIV treatment services in three countries, and identifies best practices to prevent treatment interruptions during an emergency. This is essential given that 200 million people and 30% of sub-Saharan Africans live in a state of chronic, recurrent or episodic emergency (WHO 2006). Data were obtained from over 50 key informant interviews with program managers, government officials, clinicians, and patients in Kenya, Uganda and Ivory Coast. Semi-structured, in-depth interviews were conducted in person and via telephone. Site visits to programs and clinics which provided HIV treatment during recent emergencies were also conducted. Common themes were identified to generate lessons learned. None of the study countries had national contingency plans for HIV treatment during emergencies prior to recent episodes of unrest. Informants described predictable challenges: disruptions to communication, transportation, and supply chain networks, and strain on human resources. Strategies for optimizing treatment continuity in emergencies should be developed at the policy (e.g., creating and disseminating national contingency plans for ART access during emergencies and inclusion of guidance on ART treatment during emergencies within national treatment guidelines), program (e.g., providing facilities with buffer stock and developing tracking and referral systems), and patient (e.g., establishing support systems for those who are displaced, providing patients with copies of medical records and educating patients on forced treatment interruptions) levels. In addition, responders to complex emergencies must be aware of the needs of PLWHIV. Emergencies are unpredictable but planning may optimize continuity of HIV treatment. Development of interventions at the policy, program and patient level in advance may prevent HIV treatment interruption when emergencies arise.

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### TRAINING HEALTH WORKERS TO IMPROVE QUALITY OF CARE: DEVELOPMENT OF A THEORY-BASED TRAINING PACKAGE IN PATIENT-CENTERED SERVICES AND HEALTH CENTER MANAGEMENT IN UGANDA

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Universal access to appropriate malaria case management is advocated by the World Health Organization and others to reduce malaria morbidity and mortality in low income settings. However, increasing access to services has proven challenging and the evidence base is poor. In eastern Uganda, care seekers are discouraged from attending public health facilities due poor health center management, frequent drug stock-outs, limited skills and motivation of health facility staff, and poor relationships between health workers and communities. A large cluster randomized trial, the PRIME study, is evaluating a multi-faceted health facility-based intervention to address these shortcomings in Tororo, Uganda. Field activities began in December 2010, and the intervention was rolled out in May-June 2011. Study follow-up will continue until April 2013. A central component of the PRIME intervention is a series of nine interactive training modules to strengthen health worker-patient interactions to be

more patient-centered and to improve health center management in line with a revised system for maintaining supplies of rapid diagnostic tests and artemether-lumefantrine. The design of such interventions is rarely presented, reflected in the poor evidence base available for program planning. The methods used to design the PRIME modules, consisting of empirical formative research in the local area, a review of evidence of other interventions, articulation of a theory-based behavior change model, and piloting of the training modules will be reviewed. The impact of these training modules on proximal outcomes at 10 health facilities randomly assigned to receive the intervention, compared to 10 assigned to continue standard care, will be presented including daily patient attendance data, availability and management of key malaria commodities, and patient satisfaction with the health facility visit.

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### WHERE TO DELIVER? INTENTION VERSUS PRACTICE IN PREGNANT WOMEN IN SOUTHERN PROVINCE, ZAMBIA

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In Zambia, 94% of pregnant women have at least one antenatal clinic visit, 52% of deliveries occur at home (72% in rural areas), a skilled health worker attends 47% of deliveries, and the maternal mortality ratio is 591 deaths/100,000 live births. Recently, the Zambian Ministry of Health has promoted facility-based deliveries with the goals of lowering maternal mortality and improving birth outcomes. Pregnant women were recruited at 90 health centers (HCs) during routine antenatal care in Southern Province, Zambia to participate in a large neonatal survival trial (ZamCAT). Enrolled women were asked where they planned to deliver and why, and were interviewed 4 days post-delivery about the delivery and reasons for any change of location. The 9,816 respondents had mean ( $\pm$  SD) age of 25.8  $\pm$  6.9 y, median of 3 pregnancies, and 41% had only primary education. When interviewed antepartum, 93% of respondents intended to deliver at a HC, 6% at home, and 1% had no plan. However, 63% delivered in HCs, 36% at home and 0.9% elsewhere. Of those who intended to deliver at a HC, only 66% actually did. In contrast, 87% of women that planned to deliver at home actually delivered there ( $p < 0.01$ ). Women who delivered at home were older (26.8 vs. 25.1 y,  $p < 0.001$ ), less educated ( $p < 0.001$ ), and had higher parity (3.0 vs. 2.3 pregnancies,  $p < 0.001$ ). Women who intended to deliver at home and subsequently delivered at a HC stated they needed a skilled attendant, or for the safety of the mother and baby. Fifty-five percent of deliveries were attended by a nurse/midwife, 21.6% by a family member, 17.4% by a traditional birth attendant, and 3.7% self-delivered. Reasons for home delivery included distance, finances, and family/societal pressures. 12.6% of women who delivered at home gave other reasons including short duration of labor, no transportation, and timing of labor. To increase HC-based deliveries in rural areas of Africa such as this study site in Zambia, efforts need to be made to understand sociocultural barriers and to reduce costs of and facilitate transport to the HC.

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### NATURAL INFECTION OF *LUTZOMYIA EVANSI* (DIPTERA: PSYCHODIDAE) WITH *LEISHMANIA (VIANNIA) SPP.* IN NORTHERN COLOMBIA

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The most important mixed focus of cutaneous and visceral leishmaniasis in Colombia is located in Los Montes de María, a mountainous region of the northern departments of Bolívar and Sucre. Although the phlebotomine sand fly *Lutzomyia evansi* is recognized in this area as the

vector of *Leishmania infantum*, etiological agent of visceral leishmaniasis, the vectors of the parasites responsible for the cutaneous form remain unknown, motivating the search for natural infections in phlebotomines from the Montes de María area of Sucre. Flagellates were sought under the microscope in the dissected guts of sand flies captured by Shannon trap as well as daytime collections from peridomestic resting sites. The guts of infected insects were used for parasite isolation in NNN culture medium and molecular characterization by sequencing of the internal transcript spacer (ITS1) region. Two specimens of *Lu. evansi* were found infected with flagellates among 1619 captured, corresponding to an infection rate of 0.12%. Parasite distribution within the guts of infected phlebotomines corresponded to that exhibited by *Leishmania* species of the subgenus *Viannia*. Nucleotide sequence analysis allowed flagellates found in *Lu. evansi* to be grouped with reference strains and isolates of *Le. (Viannia) braziliensis* of cutaneous leishmaniasis patients resident in Montes de María. The natural infections of *Lu. evansi* with parasites of the subgenus *Le. (Viannia)* constitutes the first biological and molecular evidence of vectorial competence in this species and its possible participation in maintaining the epidemiological cycle of cutaneous leishmaniasis in northern Colombia.

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### PREVALENCE OF SCABIES IN FIJI: A NATIONAL STUDY

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The World Health Organization estimates that 300 million people worldwide are affected with scabies each year. Scabies and skin sores are recognised by clinicians and public health practitioners in Fiji and other countries in the Pacific region as significant health problems, however there are few population based data documenting the prevalence of scabies and skin sores in Fiji. We conducted a national epidemiological cross-sectional study to assess the prevalence of scabies and skin sores in collaboration with the Fiji Ministry of Health. The study enrolled 13,294 participants across all age groups and ethnicities. A total of 96 sites, including villages, settlements and schools were selected. The sample was representative of the national population of Fiji, particularly in regards to ethnic and age groups in Fiji. The all-ages prevalence of scabies was 23.3%. The prevalence of scabies varied by age group; there was a peak in children aged 4-7 years (51.1%), and the prevalence was also high in the children aged three years and younger (36.7%). However, no age group was free of scabies. Bacterial infection of scabies was common with 20.7% of patients with scabies having evidence of super-infection. In conclusion, this is the first national prevalence study of scabies worldwide that we are aware of. Scabies is a very common skin disease in all age groups in Fiji, and is particularly common in young children. We believe that this is likely to be the case in other tropical nations in the Pacific region, affecting up to 50% in this age group. Our data indicate that scabies has been underestimated as a tropical disease. A comprehensive and well-coordinated scabies elimination program is urgently needed. A study to assess the efficacy of a mass drug administration program is planned, comparing oral and topical treatment regimens. We aim to find the most appropriate and cost-effective solution to justify the investment of time, manpower and money to reduce the prevalence of scabies in Fiji and other tropical countries where scabies is endemic.



## IMMUNOMODULATION AT THE TICK-HOST-VIRUS INTERFACE

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Emerging and re-emerging diseases transmitted by blood feeding arthropods are significant global public health problems. Ticks transmit the greatest variety of pathogenic spirochetes, rickettsiae and viruses of any blood feeding arthropod. Infectious agents transmitted by ticks are delivered to the vertebrate host together with saliva at the bite site. 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, we have characterized tick borne encephalitis virus (TBEV) infected tick induced changes in cutaneous immune response at the early stages of attachment/feeding by *Ixodes ricinus* adults. Our preliminary analysis reveals that ticks (tick saliva) create an inflammatory environment at the bite site during the first 12 hours of feeding. Genes involved in neutrophil recruitment and migration were observed to be upregulated. We are currently investigating the immune cell recruitment at the bite site during the first 12 hours, and possibly identify the primary target cells for TBEV infection. Our study will advance the understanding of the immunomodulation at the tick-host interface induced by tick saliva that facilitates TBEV transmission and dissemination.

## DEVELOPMENT OF A MOLECULAR TAXONOMIC KEY FOR THE IDENTIFICATION OF SCRUB TYPHUS VECTORS, MITES WITHIN THE GENUS *LEPTOTROMBIDIUM*

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Larval trombiculid mites (chiggers) are important vectors of scrub typhus within Thailand and much of Asia. Identification of mite species is extremely difficult and we have proposed to develop a molecular taxonomic-key for the precise identification of trombiculid mites using the Cytochrome oxidase subunit I (COI) gene, 16S rRNA and 12S rRNA of mitochondria. Our aim to develop mtDNA barcoding is to identify the pre-defined species of mites collected from field sites, focusing mostly on the reservoir mites for scrub typhus. The evolutionary relationship among chiggers collected from wild-caught rodents trapped from different parts of Thailand was analyzed from the full-length COI gene (amino acid and nucleotide sequences), 16S rRNA, and 12S rRNA sequences. The maximum likelihood (ML) and neighbor joining methods using MEGA5 program were used to analyze the evolutionary relationship among field chiggers and the reference sequences retrieved from GenBank database. The Phylogenetic trees constructed from 12S and 16S sequences were supported by high bootstrap values on each cluster. These two sequences would probably be more suitable for chigger species differentiation. However due to the paucity of available gene sequence some species of chigger cannot be identified. Comparison of multiple DNA sequences and morphological ID will enable us to adjust and develop more accurate/rapid molecular assays for chigger species identification in the near

## IGM ANTIBODY RESPONSE OF GUINEA PIGS TO SALIVARY PROTEINS OF *TRITOMA INFESTANS*

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Salivary proteins of triatomines like of other hematophagous arthropods, injected into the host during feeding, elicit a humoral immune response in their host. This immune response may indicate a recent exposure to triatomine bites and can be a potential measure of transmission risk of Chagas disease. Therefore in this study, IgM antibody responses of guinea pigs to salivary proteins of *Triatoma infestans* were analysed. Eighteen guinea pigs were exposed to low numbers of nymphal (n=5) or adult *T. infestans* (n=5) of three different strains from Chile, Argentina and Bolivia over a period of 10 weeks. The animal sera were tested for their IgM-antibody reactivity to crude *T. infestans* saliva. In ELISA assays, IgM responses were already detected after the first week of exposure to bug bites and they increased only slightly over time during the long term exposure study of guinea pigs to triatomines. Overall, IgM antibody levels of guinea pigs were lower in comparison to IgG antibody levels of guinea pigs analyzed in previous studies. Guinea pig sera from the long term exposure study were also used to probe for salivary proteins of *T. infestans* on Western blots in order to analyze the development of IgM anti-saliva specific immune responses. Guinea pig sera recognised salivary proteins with strain and developmental stage specific variations. Despite these variations, a 35 kDa antigen was detected by sera of almost all challenged guinea pigs. This antigen was also recognized by IgG antibodies analyzed in previous studies and may be a candidate exposure marker to detect triatomine bites. Because different triatomine species are capable of replacing *T. infestans* after vector control measures, the cross reactivity of immune responses to salivary proteins of different triatomine species were examined.

## GENETIC AND PHYLOGENETIC ANALYSIS OF SOUTH KOREAN SACBROOD VIRUS ISOLATES INFECTED IN HONEY BEE (*APIS CERANA*)

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Climate change will lead to movements of honey bees of different species and races, bringing them into contact with pathogens with which they have never co-evolved. Sacbrood virus (SBV) is one of the most dangerous viruses infected in honey bee and causes the failure to pupate and death in larvae and adult bee. Genetic analysis of SBV infected honey bee (*Apis cerana*) sampling from five different provinces, South Korea in 2010 was firstly carried out based on three nucleotide sequences of a partial structural protein coding sequence and two non-structural protein coding sequences. Sequences amplified by three specific primer pairs were aligned and compared with reference sequences deposited in GenBank database. Sequence alignments revealed a low level of sequence variation among Korean isolates (with  $\geq 98.6\%$  nucleotide identity), regardless of the genome regions used and geographic origins of the strains. Multiple sequence comparisons indicated that the investigated Korean SBV isolates are genetically closely related to Chinese and other Asian strains. Especially, Korean SBV isolates showed the numbers of private nucleotides and amino acids, which were not ever observed in other published strains. Korean isolates and a Chinese isolate (assigned as AF469603) hosted by *A. cerana* and a Chinese strain (assigned as HM237361) hosted by *A. mellifera* showed the difference in nucleotide and deduced amino acid identities. It strongly suggests that the host-specificity could be occurred among SBV strains isolated from different hosts. Phylogenetic relatedness between compared sequences was analyzed by MEGA 4.1 software using

neighbor-joining (NJ) method with boot-strap value of 1,000 replicates. Obtained topologies were in agreement with previous studies, in which, distinct groups of SBV were formed by a group of UK and European genotypes and a group Asian genotypes comprising strains originated from China, India and Nepal. However, phylogeny based on partial protein structural coding sequence grouped all Korean SBV isolates infected *A. cerena* as separate cluster. Our finding suggested further study including the Korean SBV isolated from *A. mellifera* is needed.

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### IDENTIFICATION OF BLOODMEALS OF *LUTZOMYIA EVANSI* (DIPTERA: PSYCHODIDAE) IN RURAL AND URBAN ENVIRONMENTS OF NORTHERN COLOMBIA

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The methodologies traditionally used to identify wild and domestic reservoirs of *Leishmania* may require arduous fieldwork and produce a considerable ecological impact on the study areas. Among the new research alternatives generated to overcome these limitations is the use of molecular biological techniques to determine bloodmeal sources of phlebotomine sand fly vectors of *Leishmania* spp., thus helping to identify the vertebrate reservoirs of the parasite without the need to sacrifice large numbers of animals. In the present study a 358-bp segment of the mitochondrial cytochrome b gene was sequenced to identify the blood meals of *Lutzomyia evansi*, recognized vector of *Leishmania* spp. in the north of Colombia. Phlebotomines were collected in the rural area of Los Palmitos and Coloso, and the urban area of Ovejas, in the Colombian department of Sucre, where both visceral and cutaneous leishmaniasis are endemic. Analysis of bloodmeal sources of *Lu. evansi* show that it feeds on at least 7 species of vertebrates, among which *Bos taurus*, *Equus asinus* and *Homo sapiens* represented 68,4% of the total. Among the less abundant hosts *Sus scrofa*, *Equus caballus*, *Gallus gallus* and *Proechimys guyanensis* together constituted 14% of bloodmeals, while undetermined samples made up the remaining 17,5%. Some of the species encountered have previously been identified as reservoirs of *Leishmania* spp. The epidemiological implications of these findings are discussed.

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### MICROENCAPSULATION OF AGRO-PEST BIOCONTROL AGENTS TO ENHANCE GLOBAL FOOD SECURITY

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Food security and availability are often associated with overall health quality at local and regional levels. For example, scarcity of food as the result of pestilence can be detrimental to subsistence-level farmers and consumers in poverty stricken regions of sub-Saharan Africa. Current strategies for controlling agricultural pests rely heavily on chemical pesticides. Pesticide resistance, environmental toxicity and unintended health consequences, nonetheless, are emerging issues. Biologically-based pest control agents have garnered much attention - *Metarhizium anisopliae* is highly effective in killing desert locusts (*Schistocerca gregaria*); *Bacillus subtilis* is being used to treat the agropathogenic fungus *Verticillium dahliae* while *Pantoea agglomerans* is used to target the fire-blight causing *Erwinia amylovora*. However, all these bio-control agents suffer from a high sensitivity to UV light, necessitating repeated applications and increased expense. In this work, we propose to package these bio-control microorganisms within an alginate polymer matrix to increase their resistance to UVC. Encapsulated cells in both "wet" and "dry" alginate microspheres resulted in significantly greater survival after extended UV irradiation compared to free cells in suspension. The inclusion of a high-carbon dye further increased UV resistance under

"wet" conditions but did not augment protection under dried conditions. The concentration of carbon dye (0.01% - 5% v/v) directly correlates with cell viability following UV irradiation. However, dye concentrations greater than 5% were found to negatively impact overall cell survival. Increasing dye concentration had no effect on either microsphere shape or size. To examine a novel spray formulation, microspheres were incorporated into a guar-based resin. This resin further enhanced UV resistance in the "wet" state but did not significantly alter UV resistance in the dry state. These results represent a significant breakthrough in the use and implementation of bio-control agents in agricultural pest control.

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### ECONOMIC EVALUATION OF AREA-WIDE PEST MANAGEMENT PROGRAM TO CONTROL ASIAN TIGER MOSQUITO IN NEW JERSEY

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Area-wide pest management (AWPM) is recommended to control urban mosquitoes, such as *Aedes albopictus*, which limit outdoor activities. While several evaluations of effectiveness exist, information on costs is lacking. Economic evaluation of such a program is important to help inform policy makers and obtain appropriate resources. We conducted an economic evaluation for an AWPM in Mercer and Monmouth counties, New Jersey, as part of a controlled design (AWPM vs. control). The study analyzed financial documents and time allocated by staff implementing the programs in 2009 and 2010. Also, random samples of households in AWPM and control areas were surveyed each fall since 2008 by a combination of mailed, telephone and in-person interviews. Sample sizes in data available ranged from 311 in 2008 to 396 in 2010. Hours lost were differences between actual and potential hours of yard and porch activities in an average summer week if there had been no mosquitoes. Net estimated benefits of AWPM were based on a difference-in-difference analysis (between years and areas). Reductions in hours lost were valued based on respondents' willingness to pay (WTP) for a hypothetical extra hour free of mosquitoes that they could spend in yard or porch activities. The share of residents in AWPM areas reporting mosquitoes as a major nuisance decreased from 68.6% in 2008 to 46.0% in 2010. The net impact was an 11.6% reduction in mosquitoes nuisances ( $p=0.11$ ). Numbers of hours lost per week due to mosquitoes in AWPM areas between the base year (2008) and the second intervention year (2010) declined (mean $\pm$ SEM) by 1.88 $\pm$ 4.19 hours/week in intervention areas compared to control areas, indicating potential program effectiveness ( $p=.32$ ). This translated to 24.4 hours gained over the 13-week summer. Residents' WTP averaged \$2/hour (range: \$1-\$3), indicating a monetary valuation per resident of \$49/year (range: \$24-\$73). The average per capita expenditure on AWPM was \$30/year. These give a net benefit per resident of \$19/year (range: -\$6-\$43) and a benefit-cost ratio of 1.63 (range: 0.81-2.44). The program had favorable behavioral impact. The benefit-cost analysis of data to date suggests a positive net benefit of the AWPM from residents being able to enjoy more time on porches and yards.

### EFFECTIVENESS OF THE AREA-WIDE PEST MANAGEMENT PROGRAM TO CONTROL ASIAN TIGER MOSQUITO IN NEW JERSEY: EVIDENCE FROM A HOUSEHOLD SURVEY

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Households' behaviors can both mitigate and measure the spread of urban mosquitos. Beginning in 2009, a comprehensive area-wide pest management (AWPM) project to control *Aedes albopictus* was implemented in 4 areas in Monmouth and Mercer Counties, New Jersey. Including other activities, the project focused on increasing residents' awareness, knowledge, and mosquito control practices. Evaluating the impact of this component is important to guide future AWPM programs. We analyzed household surveys conducted in the baseline year, 2008 (310 households), and second intervention year, 2010 (396 households) in AWPM and control areas. We measured changes in hours and mitigation expenditures (e.g., repellents) from 2008 to 2010 in AWPM areas and compared results to corresponding changes in control areas. The average proportion of potential outdoor hours lost due to mosquitoes in intervention areas decreased ( $\pm$ SEM) from 29.7% $\pm$ 2.6% in 2008 to 24.3% $\pm$ 2.1% in 2010. Findings showed a net improvement of 7.0% $\pm$ 4.3% or an additional 1.89 $\pm$ 1.9 hours spent in porch or yard activities due to AWPM ( $p=0.10$ ). The share of residents bothered by mosquitoes in AWPM areas decreased from 68.6% in 2008 to 46.0% in 2010, with a net reduction in mosquitoes' nuisance of 11.6% ( $p=0.11$ ). The percentage of respondents who reported cleaning their gutters in the last 12 months increased from 21.2% $\pm$ 3.5% in 2008 to 49.8% $\pm$ 3.4% in 2010, with a favorable net impact of 9.8% $\pm$ 7.0% of AWPM ( $p=.08$ ). The AWPM had favorable net reduction of 7.0% $\pm$ 5.3 in the share of households storing tires ( $p=.09$ ) and a highly significant net increase of 20.4% $\pm$ 7.5 in the percentage of households who correctly reported the maximum number of days allowed to remove standing water to avoid breeding mosquitoes ( $p<.001$ ). Analyses through 2010 found no statistically significant impact on expenditures. Nevertheless, the project has been effective in reducing the nuisance caused by urban mosquitoes and had a favorable impact on knowledge and several yard and porch activities. Data for 2011, to be added, will provide longer term impacts.

### FILARIAL NEMATODE INFECTIONS IN *AMBLYOMMA AMERICANUM* TICK POPULATIONS IN FAIRFAX COUNTY, VIRGINIA

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Filarial nematodes are associated with a number of public health problems and cause significant morbidity and mortality throughout the world. Arthropods often serve as intermediate hosts for these nematodes and pass them to their definitive host, often resulting in disease. Mosquitoes and blackflies vector the most well known filarial nematode infections, but ticks have been known to carry veterinary filarial nematodes. Recently, we discovered *Amblyomma americanum* tick populations in Maryland infected with filarial nematodes. This discovery was the first of its kind in the Mid-Atlantic and warranted further investigation. Ticks were collected at a single site in Fairfax County, Virginia between May and September 2011 via drag method and CO<sub>2</sub> traps. Collected ticks were then sorted by species and screened for filarial nematodes using PCR methods. Filaria

amplicons were sequenced and analyzed to determine their association with known species. A total of 10 of 1223 (0.82%) *A. americanum* were positive for filarial nematode infections. Phylogenetic analysis revealed close associations with nematodes of the genera *Monanema*, *Acanthocheilonema*, *Dirofilaria*, and *Onchocerca*, which have been associated with zoonotic infections, suggesting that the filarial nematodes in Fairfax County tick populations could potentially be pathogens to humans. This initial data provides evidence that filarial nematode infections in tick populations may be emerging in the Mid-Atlantic region and increased surveillance is warranted to better characterize the nature of these infections.

### VECTORSURV AND THE RASTER CALCULATOR: TWO RECENT ENHANCEMENTS TO VECTORMAP FOR ESTIMATING VECTOR-BORNE DISEASE RISK

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VectorMap ([www.vectormap.org](http://www.vectormap.org)) is a free resource for mapping collection data and distribution models of arthropods (mosquitoes, ticks, sand flies, mites) and vertebrate reservoirs involved in disease transmission. Collection data are comprised of vouchered specimen information, and both unpublished and unpublished observation data, and are quality controlled for geographic and taxonomic information. Users can view the locations of past vector collections, the results of models that predict the geographic extent of individual vector species, and various disease distribution layers, all in a GIS-type setting. This presentation focuses on two enhancements to VectorMap that have recently been developed: the Raster Calculator (RAC) and VectorSurv. The RAC allows the quantification, for any area of interest, of the overlap of models predicting the extent of vectors, hosts, and disease, to generate an estimate of hazard and risk. The RAC approach emphasizes the interactions and independent contribution to disease risk of the vector, host, and pathogen species predicted present. VectorSurv is designed for online reporting of regular surveillance data and allows the user to understand when vector population densities are above average, by providing information-rich graphs of species seasonality and abundance. These resources are offered as a place for publishing vector collection data, and for exploring the nexus between geography and vector-borne disease transmission.

### NEW PHLEBOVIRUS RELATED TO SANDFLY FEVER NAPLES VIRUS ISOLATED FROM SANDFLIES COLLECTED IN TUNISIA AND ITS POTENTIAL IMPACT ON PUBLIC HEALTH

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Sandflies are widely distributed around the Mediterranean. Therefore, human populations in this area are exposed to sandfly-transmitted diseases, including those caused by phleboviruses. While there is substantial data in countries located in the northern part of the Mediterranean basin, few data are available from North Africa. Sandflies were collected from the site of Utique from the governorate of Bizerte located in Northern Tunisia, a well-known site of visceral leishmaniasis, during the summers of 2008, 2009, and 2010. In 2008 and 2009 sandflies were captured and pooled by sex and species. *Phlebotomus perniciosus* is the most abundant sandfly species (75%). Thus species identification was abandoned in 2010 and sandflies were pooled by sex. Sandflies were tested for the presence of phleboviruses by PCR. Viral RNA corresponding to a novel virus closely related to Toscana virus was detected in pools of sandflies collected in 2008, 2009, and 2010. Virus isolation in Vero

cells was achieved. Genetic and phylogenetic characterisation based on sequences in the three genomic segments showed that it was a novel virus distinct from other recognized members of sandfly fever Naples virus complex. This novel virus was provisionally named Punique virus and transmitted mainly by *P. perniciosus*. To study the impact of this virus in term of public health in Northern Tunisia, a sero-epidemiological study was performed by Virus Neutralization Test using Toscana virus for the screening stage (dilution 1/10 to 1/80). Positive sera were then tested concomitantly against Toscana and Punique virus by using two-fold dilutions from 1/10 to 1/2560 to discriminate between the two viruses. The results of the screening step indicated that a large percentage of the population had antibodies capable to neutralize Toscana virus (40.7%: 516/1266). The second stage will allow us to determine whether Punique virus is able to infect humans.

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### DIVERSITY, DISTRIBUTION, AND ABUNDANCE OF MEDICALLY IMPORTANT SAND FLIES (DIPTERA:PSYCHODIDAE, PHLEBOTOMINAE) IN THE SOUTHERN PERUVIAN AMAZON BASIN

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*Lutzomyia* spp. are known to transmit leishmaniasis and bartonellosis throughout South America; however, information about sand fly distribution, abundance and vector potential is limited. Moreover, construction of an interoceanic highway in South America could have had an effect on the diversity and distribution of medically important sand flies. Herein, we examine habitat perturbation impacts on sand fly diversity and abundance by means of a survey conducted in six sites located along the interoceanic highway in the southern Peruvian Amazon Basin: Iberia and La Novia (Tahuamanu, Madre de Dios); Florida Baja, Alto Libertad, and Mazuko (Tambopata, Madre de Dios); El Carmen (Carabaya, Puno). Study sites were divided into 200, 600 and 1000 m transects along both margins of the highway. Sand flies were collected using CDC light traps, Shannon traps and protected human baits. A total of 7,381 sand flies were identified to two genera (*Lutzomyia* and *Brumptomyia*), 9 sub-genera, and 49 species, with 1,550 (21%) males and 5,831 (79%) females. CDC light traps, Shannon traps and protected human baits captured 3,894 (53%), 3,461 (47%), and 26 (0.4%) sand flies, respectively. The Shannon-Weaver diversity index (H) was higher in collections with CDC light traps (H=1.23) and Shannon Traps (H=1.01) than with human bait (H=0.67). The six most abundant collected species are known to transmit leishmaniasis in other Amazon Basin countries, and considered potential vectors in Peru: *Lutzomyia carrerai* (20%), *Lu. davisii* (14%), *Lu. richardwardi* (13%), *Lu. yucumensis* (11%), *Lu. shawi* (9%) and *Lu. hirsuta* (3%). Sites in Tambopata had the highest species diversity in this study: Alto Libertad (H=1.1), Florida Baja (H= 0.9), and Mazuko (H=0.9). Interestingly, 600 m transects (H=1.09 right margin; H=1.11 left margin) and 1000 m transects (H=0.97 right margin; H=1.22 left margin) had a higher diversity index than 200 m transects (H=0.81 right margin; H=0.83 left margin). Potential vectors represented 90% of the sand fly species in the 200 m transect, and 78% and 86% in the 600 m and 1000 m transects, respectively. This study provides information about sand fly species diversity, distribution and abundance, and suggests a widespread distribution of sand fly species that are known leishmaniasis vectors in the Amazon Basin. Future studies will determine *Leishmania* infection rates of these captured sand flies to predict and prevent disease transmission potential.

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### MUTATIONS OF THE MEXT GENE IN PSEUDOMONAS AERUGINOSA ISOLATES ARE NOT ASSOCIATED WITH MULTIDRUG-RESISTANCE

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*Pseudomonas aeruginosa* is characterized by its ability to use multiple mechanisms to become resistant to several antibiotics. The extrusion of antimicrobials mediated by active efflux pumps represents one of them, with *P. aeruginosa* having 11 different known efflux pump systems. Among these, MexEF-OprN which is quiescent in *wild-type* strains *in vitro*, is over expressed in *nfxC*-type mutants, conferring resistance to quinolones, trimethoprim, chloramphenicol and imipenem. The *mexEF-OprN* expression in *P. aeruginosa* typically results from mutations in the *mexT* gene (positive regulator of this specific operon) and is associated with multidrug-resistance (MDR). From August 2011 through February 2012, 52 clinical isolates of *P. aeruginosa* were collected from Daniel A. Carrion Hospital in Lima, Peru and tested for drug susceptibility (*nfxC* phenotype) through disk-diffusion methods per Clinical and Laboratory Standards Institute (CLSI) guidelines. The *mexT* gene was amplified by PCR and mutations within it were detected by comparing sequencing data according to the PAO1 *P. aeruginosa mexT* gene reference sequence from the NCBI (National Center for Biotechnology Information). 45 isolates (85.6%) showed MDR to 3 or more antibiotics. The *nfxC*-type phenotype was found in 44 isolates (84.6%). 49 of the 52 amplified DNA samples were correctly sequenced and all were shown to have 8 consecutive deletions starting in the 105<sup>th</sup> position. Moreover, 20 punctual mutations were found throughout the sequences. 18 were synonym-type mutations and 2 were non-synonyms. There were 14 combinations of mutations arranged in 14 different genotypes. 9 genotypes were present among 42 of the MDR isolates and 5 genotypes present among 7 of the susceptible isolates. In conclusion, consecutive deletions at the 105<sup>th</sup> position and noted punctual mutations within the *mexT* gene were present in both sensitive and MDR isolates and are not directly associated with multidrug resistance.

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### IN VITRO EVALUATION OF TWO HERBAL FORMULATIONS AS ANTI-INFECTIOUS MEDICINAL AGENTS

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This study evaluated the *in vitro* antimicrobial activity of two herbal formulations (A and B) against standard (n=7) and clinical bacterial (n=16) isolates using the agar-well diffusion method. In addition, the possible *in vivo* toxic effects were studied using Sprague-Dawley rat and the following phytochemicals, alkaloid, flavonoids, polyuronides, reducing sugars, cyanogenic glycoside, saponins, terpenes, anthracenosides, phytosterols and phenols screened. Formulation-A, inhibited the growth of 67% of the Gram-positive bacteria used while only 7% of the Gram-negative bacteria was inhibited. In total, Formulation-A inhibited the growth of a total of 23.8% of the microbes used. Formulation-B, inhibited all the Gram-positive bacteria and 60% of the Gram-negative bacteria used. In total, it inhibited the growth of 62% of microbes used. In the MIC analysis, Formulation-A exhibited minimum inhibitory concentrations ranging from 0.5 to 16.0 mg/ml for the standard strains whilst the wild strains had MICs ranging from 4.0 to 32.0 mg/ml. In the case of the Formulation-B, the MICs ranged between 1.0 and 2.0 mg/ml for the standard strains while for

the wild strains it ranged from 2.0 to 8.0 mg/ml. The study also revealed the presence of saponins, reducing sugars, phenolics, polyuronides, and triterpenes as the major phytochemical components of both formulations with alkaloids and flavonoids present only in the Formulation-B whilst phytosterols were only present in the Formulation-A. The LD<sub>50</sub> value both formulations was greater than 5000 mg/kg, making both herbal medicinal products practically non-toxic. Thus, these formulations could be developed with further research into a potent antimicrobial.

## 591

### PREVALENCE OF RICKETTSIAL INFECTIONS AMONG FEVER PATIENTS IN HENAN PROVINCE, CHINA

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Rickettsioses are among the most important emerging and reemerging infectious diseases worldwide. Henan province, located in east-central China, is a newly determined endemic area for rickettsial diseases with limited knowledge as to the prevalence of the diseases and the identity and distribution of the rickettsial pathogens. A total of 291 serum samples were collected anonymously from patients presenting with acute febrile illness at the Fever Clinic of Zhengzhou University People's Hospital from August 2011 to March 2012. Whole cell antigens were used in enzyme-linked immunosorbent assays (ELISA) testing for specific antibodies (IgG) against spotted fever group (SFG), typhus group (TG) and scrub typhus group (STG) rickettsiae to determine the prevalence of exposure to rickettsial pathogens among febrile patients in Henan. 164 of 291 (56.4%) serum samples were collected from individuals who resided in the central areas of Henan Province in and surrounding Zhengzhou city, 66 (22.7%) were from eastern Henan, the rest 61 (21.0%) were from other areas. Overall 26.7% (52/195), 14.3% (26/182) and 4.7% (10/211) serum samples of the fever patients showed reactive antibodies against TG, SFG and STG rickettsiae, respectively. The exposure to SFG and STG rickettsial pathogens was less in urban (9.9% and 1.3%, respectively) but was more in rural areas (17.1% and 7.8%, respectively), while similar exposure levels were observed among fever patients from urban and rural areas to TG rickettsiae. TG and SFG rickettsial infections were found among fever patients from all locations in Henan Province, while seroreactivity to STG rickettsiae was only detected among individuals from eastern and south-eastern areas. The infection rates of male and female patients for TG and SFG were similar, while the infection rate for STG rickettsiae was higher in female patients (7.2%) than that in male patients (3.1%). This preliminary surveillance study revealed that rickettsial infections were not rare events among a fever patient population from Henan Province, east-central China. Physicians, residents and visitors should be aware of rickettsial diseases in this area. Future studies should include identification of specific rickettsial pathogens within the TG, SFG and STG responsible for the infections.

## 592

### INCREASED RESISTANCE TO AZITHROMYCIN IN *E. COLI* FOLLOWING MASS TREATMENT FOR TRACHOMA CONTROL IN RURAL TANZANIA

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Mass drug treatment with azithromycin (MDA) in trachoma endemic communities is part of the WHO-endorsed 'SAFE' strategy trachoma control programs. MDA has been shown to lower the prevalence of trachoma and lead to short-term reductions in other bacterial infections.

However MDA can also lead to increased carriage of azithromycin resistance. In the context of a MDA program, we prospectively monitored azithromycin resistance in fecal *E. coli* collected from young children in 8 rural Tanzanian villages participating in the PRET+ Study. Four of the villages received MDA and 4 control villages did not. Rectal swabs were collected during cross-sectional surveys performed at baseline, 1, 3, and 6 months after MDA. *E. coli* isolated from the fecal specimens were screened for susceptibility to azithromycin using E-tests and to other antibiotics (ampicillin, amoxicillin-clavulanic acid, cotrimoxazole, ciprofloxacin, chloramphenicol, and erythromycin) using disk diffusion. The proportion of resistant isolates in the MDA and non-MDA villages was calculated for each survey; differences in proportion were compared using t tests. At the baseline survey the proportion of azithromycin resistant isolates was significantly greater in the non-MDA villages (0.19 vs 0.10, p=0.004). The proportion of azithromycin resistant isolates stayed roughly constant over the followup period whereas the increase in resistance was dramatic and sustained in the MDA villages (0.44 at the 1 month, 0.30 at 3 months and 0.22 at 6 months). The prevalence of resistance was statistically significantly higher in the MDA group at all follow-up surveys (p<0.05). In contrast, the prevalence of resistance to other non-macrolide antibiotics did not seem to be affected by MDA. Our study suggests that even a single dose of azithromycin results in significantly increased carriage of resistance over the 6 months following dosing and *E. coli* may be useful as a sentinel organism. While MDA is effective for trachoma elimination, it is not without costs; thus it is essential to monitor resistance levels in the wake of MDA.

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### DEVELOPMENT OF ELISA FOR THE DETECTION OF LEPTOSPIRA-SPECIFIC ANTIBODIES USING RECOMBINANT ANTIGENS

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Leptospirosis is caused by spirochetes of the genus *Leptospira*. It is considered to be the most widespread zoonotic disease in the world. Symptoms of leptospirosis are easily confused with a variety of other febrile illnesses (e.g., dengue and malaria) that require different treatment regimens. Currently, the microscopic agglutination test (MAT) is the standard method for the diagnosis of leptospirosis. It is not only technically complex but also time-consuming. With the publication of the whole genome sequences of several pathogenic species of *Leptospira*, hundreds of genes encoding surface-exposed lipoproteins and outer membrane proteins were identified as candidates for the development of rapid diagnostics for leptospirosis. We chose three candidates (LipL32, LipL41, and LigA) and prepared recombinant proteins. MAT-confirmed positive sera from two geographic locations (Thailand and Latin America) were used to evaluate these antigens using ELISA. The results showed that with the combination of all three antigens, the overall sensitivity was 84%. Samples from infections due to a wide range of *Leptospira* serovars were reactive with these recombinant antigens in ELISA. These results suggest that an easy to perform ELISA with recombinant antigens for diagnosis of leptospirosis is achievable.

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### CONFIRMATION OF COXIELLA BURNETII POSITIVE CLINICAL SPECIMENS: COMPARISON OF A SEQUENCING ASSAY AND A SECONDARY IS1111 REAL-TIME PCR ASSAY

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*Coxiella burnetii*, an obligate intracellular bacterium, is the causative agent of Q fever. *C. burnetii* is classified as a select agent because it is highly infectious, environmentally stable, and has been used as a biological

weapon. Acute Q fever is rarely fatal while chronic infections may have a case-fatality rate as high as 65%. Current methods for the detection of *C. burnetii* include direct, nested PCR and real-time PCR. Part of the multi-copy IS1111 element is currently used as a target for real-time PCR producing a 63-base-pair amplicon. The assay was modified to generate a larger product (208 bp) for confirmation of positive clinical samples through sequencing by designing a new reverse primer to complement the existing forward primer and probe for the IS1111 assay. Primer and probe concentrations were optimized using the 7500 Fast Dx (Life Technologies) instrument with PerfeCTa MultiPlex qPCR SuperMix (Quanta Biosciences). Ten banked positive samples were tested using the sequencing assay. Three additional primer and probe sets were designed to amplify the same multi-copy gene at various locations in the IS1111 element. The best performer was identified after optimization and used as a secondary assay for detection. The limit of detection for the assay is 1 fg DNA per reaction (~0.5 genome equivalents). Clinical specificity was assessed by testing 143 banked specimens. Real-time PCR results were in 100% agreement with banked positive DNAs and 98% agreement with banked negative DNAs. In conclusion, two sensitive and specific real-time assays have been developed that utilize the multi-copy IS1111 gene. One assay allows for DNA sequencing of the product and has been optimized to supplement real-time PCR for confirmation of positive clinical samples. A second real-time PCR assay was developed, optimized, and validated using banked specimens. The precision of the new assay is greater than the current PCR originally used to assess the clinical samples. Both assays may be used for the confirmation of *C. burnetii* in clinical samples.

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### PREVIOUSLY UNCHARACTERIZED BIOLOGICAL ACTIVITY OF THE MACROLIDE TOXIN FROM *MYCOBACTERIUM ULCERANS*, MYCOLACTONE

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*Mycobacterium ulcerans* is the etiological agent causing the neglected tropical disease, Buruli ulcer. This disease is endemic to sub-Saharan Africa and Australia, with case reports primarily occurring in children. Clinically, the disease initially presents as a painless nodule, which deteriorates radially to a necrotic ulcer with characteristic undermined edges. There is a pronounced lack of inflammatory response at the site of ulcers, with only anecdotal mention of secondary infection in the literature. The macrolide toxin produced by *M. ulcerans*, mycolactone, is accepted as the sole virulence factor and has been shown to cause apoptosis and necrosis *in vivo*, as well as possess immunomodulatory properties. We hypothesized that the notable lack of secondary infection may also be a phenomenon mediated by mycolactone. Results in our laboratory have shown that exposure to mycolactone arrests the growth of specific bacterial strains, including *S. aureus* and *S. epidermidis*. Interestingly, there are no reports of clinically isolated mycolactone-deficient bacteria, and the common consensus is that selection pressure must be present to maintain the otherwise genetically unstable megaplasmid that encodes enzymes for mycolactone synthesis. Our results demonstrate an example of this selection. We have found that recovery of pMUM001 plasmid could be achieved simply by inclusion of lysates from arrested cell types. Given this data, we speculate that mycolactone is an "accidental toxin" whose intended function is to provide *M. ulcerans* a competitive advantage in its natural environment.

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### DECISION-MAKING USING TRACHOMA SURVEY DATA IN KENYA: HOW MANY CLUSTERS ARE NEEDED FOR RELIABLE CONTROL DECISIONS?

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Mass drug administration (MDA) of azithromycin to trachoma endemic districts is a cornerstone of the SAFE strategy, which includes Surgery to correct trichiasis, Antibiotics to treat infection and Facial cleanliness and Environmental improvements to reduce transmission of the infective agent. Programs to eliminate blinding trachoma are complicated by changes in administrative boundaries prior to intervention, which may mean that previously collected survey data are not representative of new districts. The aim of this study was to explore whether existing data on trachomatous inflammation - follicular (TF) prevalence can be used to classify newly created districts and quantify the benefit from surveying further clusters. Existing TF prevalence data from 305 clusters in Kenya were used to examine the spatial characteristics of TF and conditionally simulate a 'gold standard' dataset which was used to evaluate a range of sampling scenarios. The performance of (i) existing survey data and (ii) the addition of further clusters were evaluated in terms of the ability to classify new districts according to WHO-defined MDA thresholds. Furthermore, the cost per correctly classified district was estimated with data from field surveys in Kenya and published estimates of treatment costs. Results showed that there was evidence of spatial clustering of disease risk across Kenya, which was partly attributable to large-scale climatic factors. Performance of existing data to correctly classify new districts was related to number of clusters surveyed and endemicity level, and negatively affected by proximity of district level prevalence to MDA prevalence thresholds. Increasing the number of clusters surveyed improved performance, with districts closer to thresholds requiring a greater number of clusters than low and high prevalence districts. This study suggests that the performance of existing TF data to classify newly created districts in Kenya is dependent both on the number of clusters and prevalence of TF, and thus the cost-efficiency of surveying additional sites will also vary with prevalence.

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### PREVALENCE OF MATERNAL COLONIZATION IN DHAKA, BANGLADESH

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Insecticide thermal fogging (ITF) is a tool to control vector borne diseases. It is generally assumed that ITF reduces vector density independently of housing conditions. Few studies have been focused on Sand Flies while also looking at housing characteristics. We conducted a 15 month longitudinal study that included two deltamethrin based ITF interventions in 12 of the 24 houses at Comunidad de Trinidad Las Minas, Capira, Panamá, an hyperendemic cutaneous leishmaniasis transmission village. During the study we followed sand fly (SF) abundance. We found a 50 to 80% reduction in SF density at fogged houses when compared with control houses, while controlling for seasonal changes in SF abundance associated with rainfall. We found some heterogeneities in the reductions, as abundance changed according to SF species, with *Lutzomyia gomezi*, *L. panamensis*, *L. dysponeta* and *L. triramula* reducing their density between

40% and 90% after ITF, in contrast to *L. trapidoi* whose density increased 5% after the ITF. Differences in the impact of ITF were associated with housing quality, the most precarious houses, i.e., those with features that ease insect entrance, had a disproportionately larger SF abundance, in some cases with an increased domiciliary SF density following the ITF. Our results suggest that ITF success potential to control SF density and Leishmaniasis transmission could be dependent on housing quality.

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### MULTIRESISTANCE IN CLINICAL ISOLATES OF *PSEUDOMONAS AERUGINOSA* FROM CUMANA, VENEZUELA

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Multidrug resistance in *Pseudomonas aeruginosa* (MDR-PA) is caused by the presence of intrinsic mechanisms and the acquisition of resistance genes from other bacteria. The presence of MDR-PA is of great concern because it limits the therapeutic options to treat patients. In this sense, we carried out a phenotypic evaluation of the resistance of 137 nosocomial strains of *P. aeruginosa* isolated from the general hospital of Cumana. These strains were classified according to standard biochemical tests and the antibiotic susceptibility was assessed using the Kirby-Bauer disk-diffusion assay, according to CLSI, using the antimicrobials: piperacillin, piperacillin/tazobactam, ceftazidime, cefepime, aztreonam, imipenem, meropenem, amikacin, gentamicin, tobramycin, netilmicin and ciprofloxacin. The results demonstrated that 64.5% (89/137) of the strains were resistant to at least one antibiotic. The strains were most resistant to CIP (51.1%), MEM (36.5%), IMP (35%), PIP (32.8%) and TZP (32.1%). Among the aminoglycosides, the strains were more resistant to NN (30.7%) and AN (29.9%). Of the 89 strains of *P. aeruginosa* resistant, 53 showed phenotypes that suggest the presence of mutations in the genes *gyrA* and *parC*, as well as 69 of them showed different phenotypes of resistance to beta-lactams, of which, 12 showed the presence of metallo-beta-lactamases. Results suggest that 49 strains had genes that code for aminoglycoside-modifying enzymes. Of the total of strains, 47 were MDR-PA. These results are very important to assess the presence different resistance mechanisms in the clinical strains of *P. aeruginosa* isolated and constitutes an alert for the high frequency of several mechanisms of resistance. Strategies must be designed and put in place in order to reduce the impact and the spread of these strains that can increase the morbidity and mortality in the patients and the costs for the health care system.

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### DETECTION OF RESISTANCE GENES CODING FOR BETA-LACTAMASES TYPE-1, BLATEM, IN *ESCHERICHIA COLI* ISOLATED FROM RECREATIONAL BEACHES IN NORTHEASTERN VENEZUELA

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*Escherichia coli* can transfer antibiotic resistance genes to other strains of *E. coli*, as well as to other Enterobacteric species. Resistance of environmental strains of *E. coli* has been increasing and it has been suggested as a result of human waste contamination or agricultural related contamination. Here we assess the resistance of *E. coli* strains isolated from 4 recreational beaches in the Golfo de Cariaco, Sucre state

and from 5 beaches in Margarita Island, Nueva Esparta state, Venezuela, for a period of 3 months, sampling every two weeks. We isolated and identified *E. coli* strains according standard biochemical tests and the antibacterial susceptibility was determined using the Kirby-Bauer disk-diffusion assay, according to CLSI. We detected blaSHV and blaTEM genes using PCR and the fragments of the amplified genes, as well as those of the 16S rRNA genes from the positive *E. coli* strains were sequenced. We isolated a total of 62 *E. coli* strains that were sensitive to amoxicillin-clavulanic acid, ampicillin-sulbactam, cefoxitin, ceftazidime, ceftriaxone, cefotaxime, cefepime, aztreonam, imipenem and meropenem. However, 23 strains were resistant to ampicillin and cefalotin, with intermediate resistance to piperacillin. All the strains showing broad-spectrum beta-lactamases did not amplified for blaSHV gene but amplified for blaTEM. Sequencing of these fragments showed that all of them were type 1 blaTEM. The sequences of the 16S rRNA showed that the strains that were isolated in the same beaches at different times were identical, but those strains isolated from different beaches showed nucleotide differences among them. This study demonstrates the presence of resistance genes coding for type-1 blaTEM in environmental strains of *E. coli*, whose origin can possibly be trace to human activities, which has implications for the transmission of these genes to the marine bacterial ecosystem.

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### PYROSEQUENCING BASED ASSESSMENT OF THE BACTERIAL COMMUNITY STRUCTURE IN TICKS

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Ticks are important vectors for a variety of diseases. There is evidence that rickettsia may compete for the same niche within ticks. To better understand the tick's role as vector, we evaluated the microbiome of *Ixodes scapularis* ticks captured in Westchester, NY, and New Haven, Connecticut. Tick DNA was extracted and purified from 20 ticks, used to generate duplicate DNA amplicons of the V1-V3 hypervariable region of the bacterial 16S rDNA gene, and subjected to 454-pyrosequencing. On average, over 5000 sequences per sample were generated. The dominant taxonomic groups across all samples (0.1% of all sequences) were Acidobacteria, Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Firmicutes Spirochaetes, and Sphingobacteria. At the genus level, the dominant genera were *Anaplasma* (5.1% reads) *Borrelia* (3.1% reads), and *Rickettsia* (26% of reads). The majority of unclassified sequences belonged to class Enterobacteriales (26%). Also, members of the genus *Rickettsia* with highly divergent OTUs were detected. The abundance of genus *Rickettsia* was higher in female ticks (average 56% reads/tick) than male (average 12% reads/tick). Further, a total of 397 bacterial genera were present in our dataset and the median number of genera found per tick was 48 (IQR: 31-123). The median concordance of genera between replicates was 91% (IQR: 79-94%).

## 601

### ENVIRONMENTAL RISK FACTORS FOR RE-EMERGING EPIDEMIC TYPHUS

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Epidemic typhus is a rodent zoonosis transmitted to man by mucocutaneous or inhalational inoculation of causative rickettsia from infected lice or humans that continues to plague refugee populations. A new zoonotic reservoir of epidemic typhus in flying squirrels was

discovered in 1975, and, since then, several cases of epidemic typhus and one of recrudescing typhus have been confirmed in patients without exposures to lice or infected humans. Forty cases of serologically or molecularly-confirmed *Rickettsia prowazekii*-caused epidemic typhus were collected over the period 1976 to 2007 and analyzed for environmental risk factors. Continuous variables were analyzed for significant differences by t-tests, and categorical variables were analyzed by chi-squares. Statistical significance was indicated by P-values  $\leq 0.05$ . Most cases of epidemic typhus occurred east of the Mississippi River ( $P = 0.00001$ ) and within the distribution range of the southern flying squirrel, *Glaucomys volans* ( $P = 0.00001$ ). There were no statistically significant differences in the gender, age, or location of residence of the reported typhus cases; but significantly more cases occurred during the winter ( $P = 0.00001$ ) than during other seasons. Case-patients were significantly more likely to report contact with flying squirrels or their nests, and to present with fever, headache, neurological manifestations, and maculopapular rash. There were no deaths. Sporadic epidemic typhus occurred in the eastern US, primarily during the winter in both rural and suburban/urban environments within the range of the southern flying squirrel and caused severe illness. Environmental health education and control strategies to reduce the likelihood of further clusters of epidemic typhus and to prevent recrudescing typhus in the eastern US should include modifying buildings and residences to prevent seasonal entry of flying squirrel colonies with their ectoparasites. The specific ectoparasitic vector(s) for flying squirrel-associated typhus has not been identified and will require further field investigations.

## 602

### MOLECULAR CHARACTERIZATION OF A NOVEL SCABIES MITE IMMUNE EVASION MECHANISM

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Scabies is a parasitic skin infestation caused by the mite *Sarcoptes scabiei*. Common worldwide, it remains a major public health problem in socially disadvantaged populations, including Australian indigenous communities. Scabies lesions are commonly co-infected with opportunistic bacteria, causing pyoderma. Scabies mites feed on epidermal protein, including host plasma; consequently, they are exposed to host defence mechanisms. We identified multiple scabies proteins functioning as inhibitors of the human complement system, a component of human innate immunity. Among them are a multi-gene family of proteolytically inactive serine protease paralogs (SMIPP-Ss), secreted into the mite intestinal tract and released into the epidermis. Our data indicate that SMIPP-Ss prevent complement-mediated damage of the mite gut. Two recombinant SMIPP-Ss investigated in detail exerted their inhibitory action due to binding of molecules involved in the three complement pathways. Immunohistochemistry demonstrated presence of the SMIPP-Ss in the mite gut, co-localising with serum components such as host IgG and complement. A neopeptide-specific antibody recognizing the pore forming surface-bound C5b-9 complex, an indicator of complement activation, did not exceed background levels, indicating that *in situ* complement activation does not occur in the mite gut. We hypothesise that SMIPP-Ss facilitate mite survival and are attractive targets for the design of novel therapeutic agents. To understand the nature of the binding to complement factors, high resolution crystal structures (1.8 and 2.0 Å) of the two SMIPP-Ss were generated. Overlaying 30 SMIPP-S sequences on the two observed structures revealed small areas of high conservation representing possible exosites with potentially important function/s. Employing site-directed mutagenesis we are currently identifying the complement binding sites in SMIPP-Ss to determine the binding mechanism. This research may lead to the development of novel preventive and therapeutic strategies to control scabies and associated bacterial disease.

## 603

### RICKETTSIOSIS IN HUMANS PRESENTING WITH FEBRILE ILLNESS IN FOUR SOUTH AMERICAN COUNTRIES

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Rickettsioses are caused by infection with intracellular rickettsiae transmitted to humans by arthropod vectors such as ticks. Although a handful of reports describe human disease in the countries of Brazil, Colombia, and Argentina, large scale serosurveys of febrile patients are lacking in the South American region. As part of a passive surveillance network for acute, undifferentiated febrile diseases, we performed serologic analysis (ELISA for IgG) for spotted fever group rickettsial (SFGR) and typhus group rickettsial (TGR) infection on samples obtained at an initial and convalescent visit in Peru, Bolivia, Ecuador, and Paraguay. Between 2007 and 2011, 8293 febrile individuals provided an acute and convalescent sample, and we identified 572 participants with at least a four-fold increase in titer to antibodies specific for rickettsiae: 284 (49.7%) had a four-fold increase for antibodies against SFGR, 233 (40.7%) for TGR, and 55 (9.6%) for both groups of rickettsiae. No significant association was found between age or gender and incidence of rickettsial infection. The most frequent symptoms, regardless of the rickettsia group, were headache (94.8%), chills (92.5%), malaise (90.9%), and myalgia (89.2%). The proportion of febrile patients with serological evidence of SFGR and TGR was significantly higher in Peru (1.2% and 1.1%; p-values 0.002 and < 0.001, respectively) than in the other three countries. Because the largest number of positive samples came from the Amazon basin city of Iquitos, Peru, we chose this city to explore a possible association between incidence of infection and either season or climate. We found no correlation between season and infection incidence and also no correlation between precipitation and infection incidence.

## 604

### SCABIES - AN OUTBREAK IN THE OUTBACK OF AUSTRALIA

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*Sarcoptes scabiei* infections are uncommon in non-Indigenous Australians but are endemic in many remote Aboriginal and Torres Strait Islander communities in Northern Australia. Scabies underlies 50-70% of Group A streptococcal skin infections which are associated with Acute Post Streptococcal Glomerulonephritis and Acute Rheumatic Fever for which Indigenous Australians have the world's highest reported rates. Most scabies cases present with a classic infection of profuse pruritis and 5-10 mites burrowed in the skin, however a more severe refractory infection called crusted scabies manifests with thousands of mites that are highly transmissible from fomites as well as close personal contact. This study was undertaken to describe an outbreak of scabies in a remote Australian Aboriginal community in May 2011. An outbreak team was dispatched to an East Arnhem community to enhance the delivery of an ivermectin MDA after a suspected crusted scabies participant was identified in the community. The response team targeted the houses of identified household and classroom contacts in collaboration with the



local researchers who were implementing a population census for scabies and strongyloidiasis prevalence and an ivermectin MDA. Classical scabies infections were diagnosed clinically and crusted scabies from clinical and laboratory investigations. Participants were administered a stat dose of 200µg/kg ivermectin unless pregnant or their weight was <15kg. The alternative medications used were 10% crotamiton daily for 3 days or 1 application of 5% permethrin. Participants diagnosed with classical scabies received 2 treatments 2-3 weeks apart and those diagnosed with crusted scabies were referred to the local health centre for evacuation to the nearest hospital for more intensive treatment. One crusted scabies participant was identified clinically with 10 other school contacts who had classical scabies infections. There were 13 priority houses identified with 184 residents in total, a median of 14 (IQR 11-21) people per house. Of the 184 residents, 153 (83%) were screened and treated with 26(17%) having scabies lesions. In conclusion, the outbreak response took almost 2 months to complete. Community awareness of the increased scabies prevalence was high and treatment was sought after by individuals and families who were not all from the priority houses.

## 605

### CO-CIRCULATION OF MOSQUITO AND TICK-BORNE ARBOVIRUSES AMONG TICKS AND THEIR ANIMAL HOSTS IN THE PASTORAL ECO-ZONE OF IJARA DISTRICT, KENYA

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Tick-borne viruses have a significant impact on both human and animal health causing severe emerging and re-emerging infectious diseases in various parts of the world. Although we have evidence of circulation of mosquito-borne viruses in the pastoral zone of Ijara District, North Eastern Kenya, prevalence of tick-borne viruses and associated tick vectors remain unknown. This knowledge is important for planning response and control by the relevant authorities. This study aimed at determining the prevalence of tick-borne viruses circulating among ticks and/or their animal hosts in Ijara District, a pastoral zone and a major hotspot for mosquito-borne arboviruses in Kenya. A total of 1520 tick pools (10,488 ticks) were sampled from both wildlife and livestock hosts (cattle, goat, sheep, camel, lesser kudu, warthog, zebra and giraffe) at various sites in Ijara district. The sampled ticks were identified using morphological keys, processed for virus screening, isolation and identification using a combination of RT-PCR, isolation in Vero cells and sequencing of amplicons. The tick species sampled included: *Rh. pulchellus* (76.12%), *H. truncatum* (8.68%), *A. gemma* (5.00%), *A. lepidum* (4.34%), *H. marginatum* (2.24%), *Hyalomma spp* (0.92%), *Rh. Appendiculatus* (0.59%), *H. dromedarii* (0.59%), *B. annualtus* (0.53%), *A. hebraem* (0.39%), *Rh .pravus* (0.20), *D. rhinocerosus* (0.07%) and unidentified nymph (0.20%). Bunyamwera, Ndumu, Semliki forest, Thogoto, Dugbe and West Nile viruses were isolated. Most of the detected viruses are known to be primarily mosquito-borne hence these findings constitute an important observation, suggesting the potential role of ticks in amplifying and disseminating viruses of public health importance far and wide. This is a first record of mosquito-borne Semliki forest, Bunyamwera and Ndumu virus isolation/detection in ticks. The observed co-circulation of viruses among ticks may provide opportunities for genetic changes and emergence of new arboviruses.

## 606

### ANIMAL HOST SKIN ODORS INCREASE TRAP CAPTURES OF ENGORGED RIFT VALLEY FEVER VECTORS IN HOTSPOT ZONES IN KENYA

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Learning cues that could reliably be associated with identifying a resource is a strategy employed by insects including mosquitoes in order to maximize the chances of obtaining the resource. These cues include host odors which would ensure that host-seeking mosquitoes locate their hosts for a blood meal. Here, we present data to suggest adaptive value of attractive host skin odors to engorged primary and secondary mosquito vectors of Rift Valley fever (RVF) based on field captures using skin odors of susceptible hosts (cow, donkey, goat, sheep and human) for this disease in two RVF hotspots in Kenya from April to September, 2011. Carbon dioxide traps baited with animal skin odors captured significantly more engorged RVF primary (*Aedes mcintoshii* *Ae. ochraceus*/*Ae. tricholabis*/*Ae. sudanensis*) ( $\chi^2 = 60.96$ ,  $df = 5$ ,  $p = 0.04$ ) and secondary vectors (*Mansonia spp.*, *Culex spp.* and *Anopheles spp.*) ( $\chi^2 = 212.7$ ,  $df = 5$ ,  $p = 0.002$ ) than the control trap baited with CO<sub>2</sub> alone. Overall, engorged mosquitoes responses were in the order cow>goat>donkey>sheep>human. The findings suggest that, the inclusion of attractive skin odors to CO<sub>2</sub> baited traps can increase captures of engorged mosquito cohort and because of their previous host encounter, may offer the potential to improve sensitivity by increasing the likelihood of viral isolations.

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### ANTIBODIES TO SPOTTED FEVER GROUP RICKETTSIA IN DOGS FROM URBAN SITES WITH REPORTS OF HUMAN ROCKY MOUNTAIN SPOTTED FEVER IN SAN JOSE, COSTA RICA

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In the past 5 years, at least 3 cases of Rocky Mountain spotted fever (RMSF) with no history of travel to endemic areas have been diagnosed in San Jose, Costa Rica. No animals or tick species were associated with these cases at the time of diagnosis. Dogs are common in urban environments and they may be implicated in transmission cycles of rickettsiae as victims and/or amplifying hosts. In this study, the possible role of dogs in the transmission of SFG rickettsia was evaluated at sites associated with human cases of RMSF. At each site, at least 50 dogs were identified within a radius of approximately 100 m from the house related to the human case, and blood samples were drawn. If the number of dogs was low, the radius was expanded until at least 50 samples were collected. Presence of IgG antibodies to SFG Rickettsia was evaluated by immunofluorescence assays using *Rickettsia rickettsii*, *R. amblyommii*, and *R. felis* antigen. Samples were considered positive when antibody titers were equal or greater than 1:32, and serial dilutions were performed to determine an end titer in positive samples. In addition, ectoparasites from each dog were collected and analyzed by a PCR targeting a genus-specific fragment of the *Rickettsia spp.* citrate synthase gene (*gltA*). In one of the sites, serologic evidence of rickettsial infection was found in 8% of dogs (5/62), and end titles to *R. rickettsii* and *R. amblyommii* in three of them were 1:64 or greater. Antibodies to *R. felis* were also detected, although end titles were much lower than those of *R. amblyommii* and *R. rickettsii* in the same sample. Differentiation of these two species was not possible. One sample from a dog belonging to a human case diagnosed more than

two years prior to this study was positive with an end title of 1:2,048. In the areas evaluated, *Ctenocephalides felis* and *Rhipicephalus sanguineus* were the most common ectoparasites of dogs. Sequencing identified DNA of *R. felis* in fleas. These results demonstrate the occurrence of SFG rickettsia infection in dogs from urban sites in San Jose associated with human cases. Considering that *R. sanguineus* and *C. felis* are common and that they are capable of transmitting *R. rickettsii* and *R. felis* to humans, the possible role of dogs and their ectoparasites in the maintenance of these pathogenic rickettsiae in urban environments requires further investigation.

## 608

### SEVERE TUNGIASIS IN RURAL MADAGASCAR: REGRESSION OF MORBIDITY AFTER WEARING SHOES IN COMPARISON TO THE REGULAR APPLICATION OF A HERBAL REPELLENT

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Tungiasis (sand flea disease) is endemic in resource-poor communities in South America, the Caribbean and sub-Saharan Africa. Frequently, tungiasis is associated with important morbidity. In some patients the intensity of the infestation is so high, that feet are severely mutilated and the patient has difficulty in walking. During an intervention study in rural Madagascar, we identified eight individuals with severe tungiasis who were not eligible for the study, since the total number of lesions was impossible to be determined. These individuals were randomized into two groups: four received a repellent based on coconut oil and jojoba oil (the standard regimen for preventing new sand fleas to penetrate) twice daily on their feet. Four individuals received a pair of solid closed shoes. This was based on the rationale that tungiasis is a self-limiting skin disease and that shoes would protect against newly penetrating sand fleas. Over a period of 10 weeks, the participants were examined every two weeks and the severity score for acute tungiasis (SSAT- a score which encompasses all aspects of acute tungiasis-associated morbidity) and the severity score for chronic tungiasis (SSCT- a score for chronic tungiasis-associated morbidity) (Kehr et al. Parasitol Res 2007; 100:413-421) were measured. Besides, clinical pathology was documented with a digital camera equipped with a macro lens. During an observation period of 10 weeks, the SSAT decreased by 41% in the shoe group: median 19.5 (range 9-20) at baseline to 11.5 (5-16) at the end of the study. In the repellent group the SSAT decreased by 88%: median 17.5 (range 10-23) to 2 (2-4). In the shoe group the SSCT remained unchanged: median 8 (range 5-17) versus 8 (7-16), while it decreased slightly in the repellent group: median 8 (range 7-14) to 6.5 (5.5-14). Although the number of participants was very small, it can be concluded that the twice daily application of a plant-based repellent reduced extremely severe acute tungiasis-associated morbidity to an almost insignificant level. The donation of shoes also reduced severe acute morbidity, but the SSAT remained unacceptably high. We suppose that shoes, without wearing socks, only partially prevent the penetration of sand fleas.

## 609

### EXPOSURE OF OUTDOOR WORKERS IN NORTH CAROLINA TO TICK SPECIES AND TICK-BORNE INFECTIONS

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Forestry, parks, and wildlife workers have prolonged outdoor exposure, increasing their risk of being bitten by ticks and infection by tick-borne microbes. In 2011, we initiated a two-year investigation of the protection from tick bites provided by permethrin-impregnated clothing. Outdoor workers enrolled in our cohort study self-reported ticks bites and collected attached ticks. The ticks were identified to species and tested for common bacterial pathogens. The lone star tick (*Amblyomma americanum*), was the predominant species collected, accounting for 95% of the 429 ticks submitted. Other species collected included the Gulf Coast (*A. maculatum*), American dog (*Dermacentor variabilis*) and black-legged ticks (*Ixodes scapularis*). Rickettsial organisms detected in ticks will be presented. Serologic tests of blood samples obtained at enrollment (n=127) revealed that many participants had pre-existing titers against spotted fever group rickettsiae. A minimum endpoint IFA titer of 1:128 was observed in 19% of participants against *Rickettsia rickettsii*, 23% against *R. parkeri*, and 11% against *R. amblyommii*. Fewer subjects had baseline titers against *E. chaffeensis* (4%). Comparison of titers from pre and post-season serum samples indicated that several participants seroconverted to spotted fever group rickettsiae and *Ehrlichia chaffeensis* during the course of the first year of follow-up.

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### RECOMBINANT PROTEIN ANTIGENS DERIVED FROM OUTER MEMBRANE PROTEIN B OF RICKETTSIA TYPHI CAN BE USED AS DIAGNOSTIC REAGENTS

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Many different organisms are classified as rickettsial pathogens and they cause a variety of diseases. In recent years, emerging rickettsial diseases have been reported throughout the world and are a significant medical concern for local and deployed personnel and travelers. Symptoms of many rickettsial infections are easily confused with a variety of other pathogens (e.g., dengue, malaria, leptospirosis, etc.) that require different treatment regimens. In order to ensure that appropriate treatment is initiated promptly, the early diagnosis of rickettsial infections is critical. Currently, the diagnosis of rickettsial diseases relies mainly on serological methods and the immunofluorescent indirect assay (IFA) has been the gold standard for diagnosis. These serological assays require the production of whole cell antigens in BSL-3 laboratories, which are not available in many endemic areas. Therefore, recombinant protein antigens, if shown to have similar sensitivity and specificity, can replace whole cell antigens as the diagnostic agents. The outer membrane protein B (OmpB) from *Rickettsia typhi* is a known immunodominant antigen and has been shown to have good sensitivity and specificity. In this report, two recombinant antigens that encompass the mature OmpB were generated by standard molecular biology techniques and evaluated for their utility in diagnosing IFA confirmed positive *R. typhi* infected patient sera. The ELISA-based assay using whole cell antigens, partially purified OmpB fraction and recombinant protein fragments showed 98%, 90% and 80% sensitivity when analyzing a total of 177 positive samples. The less sensitive assay offered by recombinant protein antigens may be due to the lack of lysine methylation as mature OmpB is known to have various lysine residues

methylated. The improvement of the recombinant protein antigens by enzymatic methylation is currently underway in order to better mimic the natural OmpB for higher sensitivity.

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### SURVIVABILITY AND THE OPPORTUNITY FOR DISEASE TRANSMISSION: OBSERVATIONS OF THE *Ixodes pacificus*

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*Ixodes pacificus* the black-legged tick is the parasite responsible for transmitting *Borrelia burgdorferi* the bacterial agent of Lyme's disease. This apparently has become more prevalent with recent evidence of confirmed cases indicative of an increased incidence among the population in states such as Maryland, Pennsylvania, Wisconsin and Connecticut. Persons that appear to be predominantly affected include hikers, campers, residents in close proximity to wildlife areas and individuals that work in various professions including parks and natural resources personnel. Evidence suggests that conditions such as global climate change, human encroachment on wildlife habitat, and an increase in resident deer population in many locations also contribute to present favorable conditions implicated in *Ixodes* spp. dispersal. Here, we introduce the concept that the increased incidence in Lyme disease could be linked to the ability of the *Ixodes* species to survive extreme environmental conditions including reduced ambient air and blood meal. This was demonstrated through observation of ticks that were placed in an enclosed environment following removal from feeding source. Here, survival is described following removal from blood meal and ambient air for several days. However, introduction to minimal ambient air elicited visible *Ixodes* motility. This is possibly an indication of the species ability to survive under adverse conditions and certainly characteristics which might help explain the present increase in population in many communities. This phenomenon might also help to answer questions regarding a greater opportunity for infecting vulnerable hosts. This area needs further research to explain any possible genetic changes that might be aiding this survival ability, and possibly to offer further insight into time that vector and bacterium might be able to survive under those or similar extreme adverse conditions.

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### THE TEMPORAL DEVELOPMENT OF ANTIBODY RESPONSES TO *SARCOPTES SCABIEI* INFECTION IN A PORCINE MODEL: RELEVANCE TO IMMUNODIAGNOSTICS

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No immunodiagnostic tests for human scabies are currently available, and existing animal tests are not sufficiently sensitive. The recombinant *Sarcoptes scabiei* antigen Sar s14.3 is a promising immunodiagnostic, eliciting high levels of IgE in infected patients. Limited data are available regarding the temporal development of antibodies to Sar s14.3, although this is relevant from a clinical perspective. We utilised a porcine model to measure scabies specific antibody levels by ELISA, comparing Sar s 14.3 to *S. scabiei* whole mite antigen extract (WMA). Robust IgG and IgE responses to both antigens were observed. Differences in the antibody profile between antigens were apparent, with Sar s 14.3 responses detected earlier and declining more rapidly after peak infestation compared to WMA. Both antigens resulted in >90% diagnostic sensitivity

from weeks 8-16 post infection. These data provide important information on the temporal humoral immune responses in scabies and further supports the development of immunodiagnostic tests for scabies.

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### IMPLEMENTATION OF AN INTEGRATED SURVEILLANCE SYSTEM IN RVF HOTSPOTS IN KENYA: ACHIEVEMENTS AND CHALLENGES

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In 2009, a consortium of partners were funded to establish an integrated surveillance system that incorporated public health, livestock and wildlife, vector and environmental sectors to monitor the activity of Rift Valley Fever (RVF) and other arboviruses in the RVF hotspots of Ijara in North Eastern (N.E.) and Baringo in the Rift Valley provinces of Kenya. Activities included sample collection from multiple host systems and species, laboratory analysis by taxonomic, serologic, tissue culture and molecular tools. Key features of the surveillance systems included the implementation of new-generation high throughput diagnostic tools. Delays in the implementation of the high throughput diagnostic platform presented challenges but use of conventional tools served as a fall back and provided level of successes in the surveillance system. Significant progress has been made in developing multiplexable arbovirus panels for sample analysis in the coming months. Virus activity detection/isolation; We have observed Inter-epidemic activity of RVF among the sentinel herds in the two hotspot areas in Rift Valley province and in N. E. Kenya (20% to 68% seroconversion rates respectively). However, despite seasonal upsurge of RVF virus primary and secondary vector populations in these locations, RVF virus activity in vectors was below detectable levels but, during the same period, other arbovirus species circulated actively among mosquitoes and ticks in detectable levels (73 and 51 virus positive pools respectively), with viruses like West Nile, Semliki Forest, Ndumu, Sindbis and Bunyamwera being detected. There has been no evidence of RVF infection/exposure among the wildlife species sampled. However, other arboviruses have been detected in ticks taken off some of the wild and domestic animals mainly alphaviruses, bunyamwera, dugbe (and dugbe-like), thogoto and unknowns, implying participation of livestock and wildlife in arbovirus maintenance and amplification in these areas. Serosurvey of sampled human populations demonstrated circulation of some of the viruses detected in vectors among humans possibly through transmission by vectors or exposure to infected animals. This underscores the value of setting up such integrated surveillance systems for arbovirus monitoring as it provides insights to dynamics of virus transmission and spread especially within a wildlife, livestock, human and vector interface.

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### RICKETTSIA OF MILITARY IMPORTANCE: AN UPDATE

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Certainly the select agents *Rickettsia prowazekii*, *R. rickettsii* and *Coxiella burnetii* that cause epidemic typhus, Rocky Mountain spotted fever and Q fever are well known to military medical leaders and therefore are considered in their plans to protect the troops. However, currently the single most important rickettsial agent for military and civilian medical

leaders is *Orientia tsutsugamushi*, which causes approximately one million cases of scrub typhus a year within the Asia-Australia-Pacific region. The presence of this agent or closely related agents in UAE and Chile should increase the concern to military planners and world public health officials that scrub typhus is not limited to the previously described endemic area. Other new rickettsiae that should also be of concern to the DoD would be those that could have a significant impact on military missions such as *R. africae*, which in 1992 caused an outbreak of African tick-bite fever among US troops training in Botswana with an attack rate of approximately 30% and *R. felis* where 3.7% to 7.7% of fever patients in sub Saharan Africa caused flea-borne spotted fever. Thus, today not only do military medical leaders need to be concerned about previously described rickettsiae but also knew rickettsiae such *R. africae*, *R. felis*, *R. aeschlimannii*, *R. spp.* D-384, *R. raoultii*, and others.

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### SEROEPIDEMIOLOGY OF SPOTTED FEVER GROUP RICKETTSIAE IN NORTH CAROLINA

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Worldwide, a large number of spotted fever group (SFG) rickettsiae have been recognized to cause disease in humans, while many more are considered to be nonpathogenic or of unknown pathogenicity to humans. In North Carolina, Rocky Mountain spotted-fever (RMSF), caused by *Rickettsia rickettsii*, is the most commonly reported tick-borne disease. Increasing entomologic and epidemiologic evidence suggests that other species of SFG rickettsiae may account for some of the reported cases of RMSF in the southeastern US. In order to better understand SFG rickettsiae infections in North Carolina, we conducted a retrospective seroepidemiologic study on paired serum samples submitted to the North Carolina State Laboratory of Public Health (NCSLPH) between 2008 and 2010. Criteria for inclusion in the study included patients for which there were paired sera available that had been submitted to the NCSLPH for testing against *R. rickettsii* and at least one of the sera had a titer greater than or equal to 1/64. We evaluated the serologic reactivity of the paired sera to *R. rickettsii*, *R. parkeri*, and *R. amblyommii* antigens. Of the 106 pairs tested, 22 were considered seroconversions (at least a four-fold rise or drop in titer) to at least one of the three antigens. Extensive cross-reactivity was found in 7 pairs, in which a seroconversion occurred against all three antigens. The number of seroconversions that were unique to each antigen were: one for *R. rickettsii*, four for *R. parkeri*, and six for *R. amblyommii*. Three pairs had seroconversions against both *R. parkeri* and *R. amblyommii*, but not *R. rickettsii*. Although serologic diagnostic methods for SFG rickettsiae are of limited value due to cross-reactivity, these findings are suggestive that species of SFG rickettsiae other than *R. rickettsii* are causing infections among North Carolina residents.

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### A GLOBAL SYSTEMATIC REVIEW OF SCABIES AND PYODERMA PREVALENCE STUDIES

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The objective of this study was assess the global prevalence of scabies and pyoderma using surveys with uniform methodologies performed on the general population and representative of a wide geographical area, with a particular focus on developing countries. Three hundred and three surveys of the prevalence of scabies and pyoderma were found using a systematic review of MEDLINE and EMBASE from January 1985

to November 2011. Twenty-seven studies were selected for comparison based on their consistent methodological and diagnostic techniques. Only studies reporting population prevalence were included in the review. There is a high prevalence of scabies and pyoderma worldwide. The highest scabies rates recorded in the general population range between 33% and 46% in Panama and Ethiopia respectively. In each study, the scabies rates dramatically increased when only data on children were analysed. The highest scabies rates in studies involving children were recorded in Panama, with 78% of children under 2 years being affected. The Australian indigenous communities have also very high prevalence rates, reaching level between 32% and 35%. The prevalence of pyoderma recorded in these studies is also very high. In the general population, its highest prevalence ranged from 43% to 49% in the Solomon Islands and the Australian Aboriginal communities. Similarly to the trend of scabies rates, these studies clearly show that the rates of pyoderma increase in the younger populations. All studies were conducted in rural settings, except for two, where both urban and rural sites were selected and compared. In conclusion, this review includes a range of fairly diverse studies conducted around the world, but mostly in impoverished areas. Although the methods and settings may vary, the papers show very high rates of scabies and bacterial infections, particularly in children. In tropical developing countries, Sub-Saharan Africa, as well as the Australian Aboriginal communities, scabies and pyoderma appear to be highly prevalent. Although several studies have been conducted globally to assess the prevalence of scabies and pyoderma in community setting, no national study has been carried out to this date, other than the one conducted in Fiji by our research team.

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### SPATIALLY EXPLICIT MEASURES OF SEASONALITY SHIFTS: NEW METHODS TO PROVIDE QUANTITATIVE ESTIMATES OF THE SHIFT VECTOR DISTRIBUTIONS UNDER ALTERED CLIMATES WITH AN APPLICATION TO LYME DISEASE

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Disease vectors respond to altered climate through changes in both population size and seasonality, and these dynamic characteristics contribute to transmission risk. Quantitative measures to analyze climate-driven shifts in population dynamics are lacking, particularly in the spatial domain. Traditional approaches, such as climate suitability indices, provide limited information on joint spatial-temporal changes in population. Here, we demonstrate the usefulness of spatially explicit mathematical modeling to address vector population response to climate change, and provide measures of projected spatial shifts in population size and seasonality for the Lyme disease (LD) vector. We assessed spatial changes in population dynamics of *Ixodes scapularis* for a baseline time period (2001-04) and two projected time period scenarios (2057-59), RCP 4.5 and 8.5, using a temperature-forced, multi-stage population model run across an eastern US grid of 4x4km cells. Multiple measures were developed to describe and compare the absolute size and timing of population signals across cells and between scenarios. Simulated populations under current climate were assessed against CDC observed LD incidence data. The projected response of *I. scapularis* to climate scenarios showed non-uniform geographic shifts in seasonality across life stages. Questing nymph and larval stages generally showed shifts to earlier questing seasons while questing was delayed in adults. Changes in season length were also observed in projected scenarios. Peak population size and Peak month (month of peak population) showed strongest associations in all life stages with CDC observed LD. The nymph exposure measure (*IP pop*), combining population size and timing, was a stronger predictor than *IP pop* in adult and larval stages. Methods presented reveal geographically varied response to climate in questing life stages, and some measures show agreement with observed data, suggesting their utility for examining climate-driven spatial shifts in population dynamics, seasonality and consequent vector-borne disease risk.

### CLINICAL RELEVANCE OF A DENGUE LYOPHILIZED ASSAY ON THE JOINT BIOLOGICAL AGENT IDENTIFICATION AND DIAGNOSTIC SYSTEM (JBAIDS) PLATFORM

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The Joint Biological Agent Identification and Diagnostic System (JBAIDS) is a ruggedized, real-time PCR-based system that is the United States Department of Defense's Program of Record for diagnostic testing of infectious diseases of operational concern. The Walter Reed Army Institute of Research (WRAIR), in collaboration with Idaho Technology, Inc. (ITI; Salt Lake City, UT), has developed a JBAIDS assay for the detection of dengue virus (the causative agent of dengue fever) in human serum specimens. A pilot clinical evaluation of JBAIDS Dengue Fever Detection Kit was conducted using a panel of 40 positive and 20 negative archived serum specimens (as determined by viral culture). These specimens, obtained from Navy Medical Research Unit-6 (NAMRU-6) in Lima, Peru, were collected in endemic areas from patients with a clinical suspicion of dengue fever. For this study, each specimen was divided and then processed concurrently and tested independently by two methods. The first aliquot of the specimen was extracted using the Qiagen RNA Viral Kit (Valencia, CA) followed by testing with a lyophilized universal dengue virus assay produced by Tetracore (Rockville, MD). The second aliquot of the specimen was processed using the ITI 1-2-3™ Platinum Path Sample Purification Kit (ITI) followed by testing with the JBAIDS Dengue Fever Detection Kit (ITI). The Qiagen/Tetracore testing procedure was used as the independent validation assay. The Tetracore testing procedure validated all 40 positive specimens. Of the 20 negative specimens, one was found to be positive for dengue virus using the validation method and was excluded from further analysis. All of the validated dengue positive specimens were detected as positive using the JBAIDS Dengue Fever Detection Kit resulting in a positive percent agreement of 100% (40/40; 95% CI 91.2-100%). Of the 19 validated negative specimens, one was found to be positive using the JBAIDS Dengue Fever Detection Kit, resulting in a negative percent agreement of 94.7% (18/19; 95%CI 74.0-99.9%).

### PRECLINICAL EVALUATION OF VIRAL INTERFERENCE USING OPTIMIZED DENVAX-4 VACCINE VIRUSES

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DENV serotypes 1-4 cause dengue fever, dengue hemorrhagic fever, and dengue shock syndrome (DSS). The development of an effective vaccine represents an important approach to the prevention and control of this global emerging disease. Inviragen's tetravalent DEN vaccine (DENVax) consists of the live attenuated DEN-2 PDK-53 virus and three chimeras expressing the pM (premembrane) and E (envelope) structural proteins of DENV-1, DENV-3 and DENV-4 in the DEN-2 PDK-53 backbone (DENVax -1, -3, and -4, respectively). We have demonstrated the safety and efficacy of DENVax in AG129 mice, monkeys, and humans. In these studies we have identified tetravalent formulations that induce neutralizing antibodies to all four DENV serotypes. However, the responses to DENVax-4 were limited by interference from the other, more robust chimeras. A common approach to overcome interference is to test different ratios of the four DENVax components. An alternative approach is to further optimize the DENVax-4 vaccine construct through genetic manipulation. We tested

these hypotheses *in vitro* and *in vivo*. Specifically, we passaged the DENVax-4 vaccine strain in Vero cells, and identified adaptive mutations in the structural and nonstructural regions of DENVax-4. Additionally, we have reverted some of the mutations acquired by PDK-53 which do not contribute to vaccine safety and attenuation, but may impinge upon growth or replication of DENVax-4. These mutations were engineered to create several infectious clones. RNA transcribed *in vitro* from the infectious clones were used to transfect Vero cells. Ongoing characterization of the resulting DENVax variant strains includes increased viral replication *in vitro*, plaque size, and growth on mosquito cells. Additionally, we are evaluating the safety and immunogenicity of the DENVax variants in AG129 mice as mono- and tetravalent formulations. Immunogenicity of multivalent formulations will determine whether re-engineering vaccine strains and optimizing vaccine ratios can have an effect on viral interference in dengue vaccines.

### REAL-TIME QUANTITATIVE REVERSE TRANSCRIPTASE-PCR (QRT-PCR) ASSAYS TO SUPPORT DENGUE VACCINE DEVELOPMENT AND CLINICAL CONFIRMATION OF WILD-TYPE DENGUE CASES

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The Sanofi Pasteur CYD dengue vaccine in Phase III efficacy trials is a tetravalent vaccine designed to elicit immune responses against the four wild-type dengue virus serotypes. qRT-PCR assays were developed and validated to assess dengue infections in subjects enrolled in clinical trials. To facilitate processing of the large number of samples, a screening assay targeting a dengue-conserved region of the 3'-UTR (Pan-DEN) was first used to identify potential positive sera, followed by four individual serotype assays. The serotype-specific assays each target conserved sequences of the dengue NS5 genomic region. Robustness, using a split-plot experimental design, assessed the impact of changes in PCR profiles, primer, probe and master mix concentrations. All assays were extremely robust, with less than 5-6% difference for most of the parameters tested, and <10% difference for two parameters. The assays were validated for specificity, precision (repeatability and intermediate), linearity/dilutability, matrix effects (lipidic and hemolytic sera and additional sera from non-dengue vaccine trials), range, accuracy, and two different automated nucleic acid extraction systems. All five assays are linear across their full range, are very precise (SD <0.3 log GEq/ml) even at the LLOQ (Lower Limit of Quantitation). All were highly specific, exhibiting <0.1 log GEq/ml absolute difference in expected values when competed against closely related targets. Matrix has little or no effect on expected values showing <0.3 log GEq/ml absolute difference in expected results when tested against hemolytic, lipidic, or homologous patient sera. The combined use of the Pan-DEN screening qRT-PCR assay with specific serotyping assays is a sensitive and specific test algorithm to identify and virologically confirm dengue cases.

### WICKING ASSAY FOR THE RAPID DETECTION OF DENGUE VIRAL ANTIGENS IN MOSQUITOES (DIPTERA: CULICIDAE)

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There is a real threat for dengue re-emergence in many regions of the United States especially in areas where the disease vectors, *Aedes aegypti*

and *Ae. Albopictus* are readily available. Unfortunately there are currently no accurate and reliable diagnostic methods to provide critical, real-time information for early detection of dengue virus within the vector populations in order to implement appropriate vector control and personal protective measures. In this paper, we report the ability of a novel lateral flow immunochromatographic assay developed by VecTOR Test Systems Inc. to determine if a pool of female *Aedes* mosquitoes is infected with any of the four dengue virus (DENV) serotypes. The DENV dipstick assay was simple to use, did not require a cold chain, and provided clear results within 30 minutes. It was highly specific and did not react with samples to which had been added Rift Valley fever virus, Chikungunya virus, Venezuelan Equine Encephalomyelitis virus, Japanese encephalitis virus, Ross River virus, LaCrosse virus, Caraparu virus, West Nile virus, and Yellow fever virus. The DENV assay can provide rapid, easy to use assay to alert public health personnel to real-time critical information on the presence of DENV in mosquitoes. Results from this assay will allow a rapid threat assessment and the focusing of vector control measures at high-risk areas.

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### ATTENUATED DISEASE IN POST-SECONDARY DENV-3 AND DENV-4 INFECTIONS IN IQUITOS, PERU

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Antibody induced by infection with any one of the four dengue virus serotypes (DENV 1-4) can influence the clinical outcome of subsequent infections with other serotypes, either enhancing or attenuating symptoms. While enhancement during second infections has been well studied, attenuation during third and fourth infections has not been rigorously examined. We estimated rates of disease as a function of DENV infection history using serological and surveillance data collected from longitudinal dengue studies in Iquitos, Peru, over 5 years. During this period, the city experienced intense transmission of DENV-3 (2006-2008) followed by DENV-4 (2008-2011). Infection history and DENV exposure during the study period were determined by PRNT70. Cases were detected by active door-to-door surveillance for acute febrile illness. Between September 2006 and February 2011, 39% (420/1077) and 53% (1595/2997) of the susceptible sample population seroconverted to DENV-3 and DENV-4, respectively. Clinical disease was detected in 7% (51/691) of DENV-3 infections and 10% (161/1595) of DENV-4. Rates of symptomatic illness among primary and secondary infections with DENV-3 (26% and 15%) were higher than in post-secondary infections (2%). Primary and secondary infections with DENV-4 also resulted in higher rates of disease (21% and 17%) than post-secondary infections (7%). Relative to individuals with <2 prior DENV exposures, odds ratios for risk of disease during post-secondary infections were 0.048 for DENV-3 and 0.22 for DENV-4. Post-secondary infections constituted a majority of seroconversions, and disease detected in these cases, despite attenuation, still represented a substantial proportion of all cases within the study population. Preexisting antibodies provided significant but incomplete protection against symptomatic illness during heterologous third and fourth infections. These findings have important implications for interpretation of surveillance data, estimating the global pattern of dengue transmission and burden of disease, and vaccine design and evaluation strategies.

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### PHYLOGENETIC ANALYSIS OF DENGUE VIRUS TYPES 1 AND 4 CIRCULATING IN PUERTO RICO AND KEY WEST, FLORIDA DURING 2010 EPIDEMICS

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Dengue is caused by any of the four dengue virus types (DENV-1 to -4). DENV is primarily transmitted by the mosquito *Aedes aegypti* and most infections are asymptomatic or sub-clinical. In the US, dengue is endemic in Puerto Rico (PR), which in 2010 experienced the largest epidemic in its history with >21,000 dengue suspected cases, from which ≈75% were laboratory-confirmed. Autochthonous dengue transmission has occurred in Key West (KW), FL during 2009-2011. The aim of this study was to analyze the genetic makeup of DENV circulating among viremic blood donors from PR and KW during 2010 dengue epidemics. Plasma samples from 6 blood donors who tested repeated-reactive for DENV NS1 Ag in an investigational screening assay were used for further molecular testing by TaqMan qRT-PCR. Specimens were also tested for the presence of infectious virus by culture in C6/36 cells. Cell culture supernatants were tested for viral production by qRT-PCR, infectivity by focus-forming assay (FFA), and for sequencing of the envelope gene. Phylogenetic reconstructions were conducted by the Maximum Likelihood and Bayesian methods using MEGA5 and Mr. Bayes, respectively. All samples were confirmed positive for DENV RNA by qRT-PCR; 4 tested positive for DENV-1 and 2 for DENV-4. Plasma samples were infectious for C6/36 cells as determined by positive FFA and qRT-PCR results in culture supernatants. Analysis of the phylogenetic trees revealed that the three 2010 PR DENV-1 strains constitute a new lineage within genotype V, which are associated to other Caribbean and South American strains. The analyzed DENV-1 KW strain clustered with a strain isolated from mosquito pools collected in KW during 2010, and with Nicaraguan and Mexican strains. The two DENV-4 isolates obtained from PR associated with a number of strains that have circulated in the island during 1980s-1990s, indicating that this old lineage of the genotype II of DENV-4 still circulate in PR. This is the first report on the phylogeny of DENV circulating in PR and KW during 2010 epidemics.

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### PARAMETERS ASSOCIATED WITH THE DEVELOPMENT OF DENGUE SEVERE DISEASE IN VENEZUELA

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Dengue is currently the fastest spreading viral vector-borne disease in the world. Although dengue viruses cause asymptomatic infections or mild disease (dengue fever (DF)), the most difficult to manage and feared forms are dengue hemorrhagic fever and dengue shock syndrome which can be fatal. To date, there are no vaccines or antiviral treatment modalities for dengue. Therefore, it becomes imperative to identify parameters that can be used by medical personnel to determine, early in the disease, which patients may be at a higher risk of evolving to severe illness, and allow early treatment intervention. Dengue in Maracay, Venezuela, is hyperendemic with co-circulation of the 4 viral serotypes. The increment of dengue transmission in Venezuela has coincided with an increase in the incidence of severe disease which in 2010 reached nearly 10% of all

cases. In this setting, a case-case study was set up in 2010 to compare clinical and laboratory parameters in patients presenting with DF versus patients developing severe dengue. Patients of all ages and presenting with fever and dengue clinical criteria to 3 designated health centres are recruited after written informed consent. Dengue infection and serotype is confirmed by RT-PCR. If positive, the patient is followed daily with clinical examination and sequential blood sampling at determined intervals up to 30 days. Severe cases are treated in a tertiary hospital and followed daily until discharge. Viral load dynamics, hematological parameters and serum levels of selected biochemical markers, including hepatic transaminases, cholesterol, triglycerids, pancreatic amylase are determined in blood samples obtained during the acute phase of the disease. Preliminary results point towards an association of severe disease with lower levels of total cholesterol and higher levels of alkaline phosphatase and aspartate aminotransferase in this population.

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### TEMPORAL DYNAMICS OF THE TRANSCRIPTIONAL RESPONSE TO DENGUE VIRUS INFECTION IN NICARAGUAN CHILDREN

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Infection with dengue virus (DENV) can result in a spectrum of disease from dengue fever (DF) to dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). The clinical course evolves rapidly, and features of the host response associated with disease severity may be transient; differences in the response to primary (1°) and secondary (2°) DENV infection may also complicate efforts to identify predictors of disease severity. To characterize the temporal evolution of the host response in patients with 1° and 2° DENV infections, and identify features associated with clinical severity, we measured fever-day-specific genome-wide transcript abundance patterns in 105 peripheral blood mononuclear cell (PBMC) samples collected from 41 children hospitalized in Nicaragua, including 9 patients with 1° DF, 11 with 2° DF, 12 with DHF, and 9 with DSS; all but two of those with DHF/DSS had 2° DENV infections. We compared transcript abundance in patients to those in 8 healthy controls and observed that differences in patients with 1° DF were greatest on day 3, while changes in patients with 2° DF or DHF/DSS peaked on day 5 (FDR<1%). Prior DENV infection explained the greatest amount of variation in gene expression among patients and was associated with increased abundance of transcripts related to the mitotic cell cycle and B cell differentiation, and decreased abundance of transcripts associated with signal transduction and cell adhesion. This pattern was common to all patient groups, but was more pronounced and occurred earlier in patients with 2° DENV infection. There were also differences in transcript abundance associated with disease severity on day 3 that were not evident later: a set of interferon-stimulated transcripts was less abundant in patients who subsequently developed DSS compared to other patient groups (p<0.05, ranksum) and patients who developed DSS had higher levels of transcripts associated with mitochondrial function (p<0.01, ranksum). These findings demonstrate that differences in the timing and magnitude of a common host gene expression signature in DENV patients are related to both evidence of prior infection and disease severity, and highlight the dynamic nature of the early host response.

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### EVALUATION OF THE DIAGNOSTIC PERFORMANCE OF THE CDC CLINICAL CASE DEFINITION FOR DENGUE, GUATEMALA: 2009-2011

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Dengue surveillance is a major priority in tropical countries due to the threat of devastating outbreaks. Diagnosis is typically based on the detection of acute febrile illness (AFI); however, suspected dengue cases are seldom confirmed with laboratory diagnosis. We evaluated the diagnostic performance of the CDC dengue case definition (CDC-D) among patients ≥2 years of age attending the Cuilapa National Hospital or Nueva Santa Rosa ambulatory facilities in the department of Santa Rosa, a low-elevation department in southern Guatemala, to differentiate dengue from other emergent diseases. The CDC-D requires fever and two or more of the following: retro-orbital pain, headache, rash, myalgia, arthralgia, leukopenia, and hemorrhagic manifestations. Active surveillance was conducted during 2009-2011 for AFI, defined as self-reported fever or measured temperature ≥38°C that began <7 days before presentation with no other diagnosis (e.g. pneumonia or diarrhea). Leukocyte cell counts were unavailable for ambulatory cases, so we evaluated a modified CDC-D among those cases. Blood specimens were taken and tested for dengue virus by real-time polymerase chain reaction (qRT-PCR) and enzyme-linked immunosorbent assay for IgM. We defined as dengue-positive those cases with anti-dengue IgM positivity or dengue virus identification by qRT-PCR on acute blood samples. We evaluated 336 hospitalized cases, 52% of which were dengue-positive, and 84 ambulatory cases, 25% of which were dengue-positive. CDC-D in hospitalized cases had 92% sensitivity, 16% specificity, 58% positive predictive value, and diagnostic odds ratio (dOR) of 2.2 (95% CI:1.1-4.3) for dengue positivity. For ambulatory cases, the modified CDC-D had 88% sensitivity, 17% specificity, 31% positive predictive value, and dOR of 2.1 (95% CI:0.5-1-8.2). Dengue surveillance using the CDC clinical case definition would capture most dengue AFI but not exclude many dengue-negative AFI cases. Further research is needed to develop a more specific case definition.

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### CLONING STRATEGY FOR THE 5'UTR-C GENOMIC REGION OF DENGUE 2 VIRUSES

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Dengue virus infections are one of the most important public health problems worldwide. The viral genome consists of a single stranded +RNA with an open reading frame (ORF) and two untranslated regions located at both 5' (5'UTR) and 3' (3'UTR) ends of the viral genome,

respectively. The 5'UTR and 3'UTR regions regulate viral replication and polypeptide translation initiation, which make them suitable for defining these fundamental mechanisms. The purpose of the present study was to develop a cloning strategy for inserting a 300 bp fragment coding for the 5'UTR, and the first 200 nt of the capsid (5'UTR-C) of seven autochthonous and four foreign strains of dengue virus 2 (DENV-2), into the plasmid pTZ18R. The cloning strategy consisted of amplifying the DENV-2 5'UTR-C fragments by RT-PCR, linearizing the pTZ18R plasmid and ligating both molecules using T4 DNA ligase. Successful cloning of the DENV-2 5'UTR-C fragments was demonstrated by PCR of the transformed *E. coli* colonies and RFLP analysis with Bgl I. This is the first time this strategy has been used to clone the DENV-2 5'UTR-C gene segment. The resultant clones will be used to analyze the DENV-2 translation mechanism and its regulation with anti-sense molecules with potential inhibitory capacities.

## 628

### CHARACTERIZATION OF DENGUE VIRUSES OF ALTERNATIVE MORPHOLOGY

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Dengue Virus is one of the most important tropical diseases today, progressively spreading to every corner of the world. It is the causative agent for dengue fever, dengue hemorrhagic fever (DHF) and DHF/dengue shock syndrome (DSS). Interestingly, classical dengue viral particles have never been visualized in acute patient plasma in spite of de facto high viremia. Our recent results, that virus can assemble into a mosaic of structures, or virus of alternative morphology (VAM), may explain the discrepancy. Immuno electron microscopy with patient plasma concentrates suggests that virus may be present inside of nondescript microvesicles (MVs). FACS data analysis has revealed that MVs from patient blood are predominantly derived from the platelets. Interestingly, dengue viral particles have been demonstrated inside of platelets isolated from acute dengue patients. Recent experiments demonstrate that infectious virus can be recovered from CD61+ cells with megakaryocytic characteristics in bone marrow of infected rhesus monkeys. Megakaryocytic cell lines, K562 and Meg01, therefore, were used as the model to further characterize VAMs. Supernatants from these infected cells are highly infectious, and yet, no classical virions have been observed. Fractionation studies by sucrose gradient revealed that high density (1.176-1.230g/ml), or heavy, microparticle rich fractions, had the most viral antigen, viral RNA, and were the most infectious in Vero cells by co-culture assays. Importantly, these VAMs were highly infectious in primary whole human bone marrow. The presence of VAM also suggests that humans likely acquire an alternative antibody response to counterattack them. Patient serum with high antibody titer to dengue viral E antigen was utilized to evaluate the neutralization capacity to the VAMs. A four-fold difference in neutralizing titer was observed between Vero-derived virus and K562-derived VAMs. This evidence suggests that focusing on the antibody response to megakaryocyte and/or platelet-derived virus may be a better index to predict protection from future infections.

## 629

### CHARACTERIZATION OF THE HUMORAL AND B CELL RESPONSE TO MONOVALENT AND TETRAVALENT DENGUE VACCINES

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Dengue virus (DENV) is the most common mosquito-borne viral disease worldwide, affecting 50-100 million people each year. There are four distinct serotypes of DENV, denoted DENV 1-4. Primary infection with any of these serotypes results in a debilitating illness that is generally not life-threatening; however, upon secondary infection with a heterotypic DENV serotype, risk increases for developing severe disease, characterized by vascular leakage leading to hypotensive shock, which may be fatal. A tetravalent (TV) live attenuated vaccine to protect against all four DENV serotypes has been developed by Inviragen (DENVax), together with monovalent vaccines (MV) against each serotype (DENVax 1-4). Previous mouse (AG129, interferon- $\alpha/\beta$ - &  $\gamma$ -receptor deficient) studies have shown the MV DENVax-4 vaccine to be less immunogenic than the other MV vaccines as well as in the TV vaccine. To further understand the humoral and B cell response to DENVax-4 and compare it with the TV formulation and wildtype (WT) DENV-4, B cell ELISpot assays against the four serotypes are being used to quantify DENV-specific antibody-secreting cells in spleen and bone marrow single-cell suspensions harvested from immunized A129 (interferon- $\alpha/\beta$ -receptor deficient) mice. Four groups and three time points are being analyzed: single immunization with DENVax TV or DENVax-4 or WT DENV-4 1036 and a control FTA diluent immunization, harvested on days 6, 14, and 28. *Ex vivo* B cell ELISpot analysis allows the quantification of DENV-specific plasma cells, while ELISpot using *in vitro* polyclonally stimulated cells enables the quantification of DENV-specific memory B cells. In addition, DENV-specific serum avidity against all four DENV serotypes is being measured using a modified ELISA assay with urea washes, and neutralizing antibody titers against the four serotypes is being determined using a plaque reduction neutralization assay. As a result of this study, we will be able to better characterize the humoral and B cell response to MV and TV vaccines and establish the basis for future vaccine studies using our improved mouse model of DENV infection and disease.

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### SANOI PASTEUR LIVE, ATTENUATED, TETRAVALENT DENGUE VACCINE TOXICITY, BIODISTRIBUTION AND SHEDDING STUDY IN THE CYNOMOLGUS MONKEY

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Sanofi Pasteur tetravalent dengue vaccine currently in Phase III clinical trial is a combination of four live, attenuated, recombinant viruses (CYD-1 to 4). Each CYD vaccine virus comprises genes encoding the non-structural proteins and capsid of the attenuated yellow fever vaccine virus, YF-17D, and the pre-membrane and envelope proteins of a wild-type dengue virus. As part of the nonclinical safety evaluation, the toxicity, biodistribution and shedding of CYD-1-4 was assessed in cynomolgus monkeys. Cynomolgus monkeys were given one subcutaneous injection of one human dose (5 log<sub>10</sub> CCID<sub>50</sub>/serotype) of CYD-1-4 or control, and were observed for 3, 9 or 21 days. Assessment included clinical observations, body temperature and weight, food consumption, clinical pathology, immunogenicity and post-mortem examinations including histopathology. Viral load, distribution, persistence and shedding of CYD-1-4 in tissues and body fluids were evaluated by qRT-PCR. The subcutaneous administration of the vaccine was well tolerated. All vaccinated animals seroconverted by day



21. Low level of CYD virus was detected in the blood and the virus was detected mainly at the injection site and the lymph nodes following the first 9 days after the injection. There were no toxicological findings other than the expected local reactions at the injection site, which correlates with previous toxicity data. These results, together with data from repeat-dose toxicity and neurovirulence studies, confirm there is no indication of toxicological concern, including risk of viscerotropism, with the CYD dengue vaccine and that transient distribution of the vaccine is limited mainly to the injection site and the lymphoid tissues.

### 631

#### DENGUE AND INFLUENZA VIRUS CO-INFECTION INCREASES MORBIDITY, MORTALITY AND VIRAL LOAD IN WILD-TYPE MICE

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Both influenza and dengue are major public health problems worldwide. In 2009, Nicaragua experienced the largest dengue epidemic in over a decade, marked by atypical clinical presentation consisting of early onset of compensated shock and poor peripheral perfusion, as we observed in our two prospective studies of pediatric dengue in Managua. Multivariate analysis revealed only the year 2009 as a significant risk factor for this outcome, and neither DENV serotype nor clade changed. Our parallel influenza cohort study and national surveillance data showed that the pandemic of influenza A-H1N1 in 2009 overlapped for 8-10 weeks with the dengue epidemic. We hypothesized that previous or co-infection of influenza A-H1N1 and dengue virus may lead to altered immune responses and more severe disease, and initial testing indicated an increased risk of shock among children with anti-influenza A-H1N1-2009 antibodies, as measured by hemagglutination inhibition. Driven by this observation in humans, we decided to develop a mouse model to further explore the possible interaction of dengue and influenza virus infections. To do this, we established a mouse model of infection with mouse-adapted influenza A/PR/8/1934 (intranasal) and the virulent DENV2 strain D220 (intravenous) in wild-type C57/BL6 mice. In preliminary experiments, infection of both viruses within two days of each other caused increased morbidity as measured by weight loss, as well as lethality in 50% of the animals compared with sublethal infection of each virus alone. The lungs of co-infected animals displayed increased DENV titers as measured by quantitative real-time PCR. Influenza titers in the lungs are currently being quantified. In addition, we have expanded a clinical isolate of the 2009 pandemic H1N1 influenza A strain from Nicaragua, which we are currently testing in our influenza-dengue co-infection model. We plan to investigate the immunological pathway(s) involved in the interaction between the two viruses and test candidate genes using genetically deficient mice. This study will help identify targets for future therapeutic strategies for patients experiencing proximal infections of influenza and dengue viruses and may inform future vaccine strategies in endemic areas where both viruses regularly co-circulate.

### 632

#### B CELL AND ANTIBODY REPERTOIRE IN SYMPTOMATIC PRIMARY AND SECONDARY HUMAN DENGUE VIRUS INFECTIONS

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The four serotypes of dengue virus (DENV) cause the most important arthropod-borne viral diseases of humans, dengue and severe dengue, with ten of millions of cases annually and 3 billion people worldwide at risk for infection. A secondary (2°) infection with a different DENV serotype is a risk factor for severe disease, but the mechanisms by which the host immune response to DENV provides either protection or enhancement of a subsequent infection with a different DENV serotype are poorly understood. We have leveraged our ongoing pediatric hospital-based and cohort studies of dengue in Managua, Nicaragua, to study the repertoire of B cells and antibodies (Abs) present during the acute phase of DENV2 primary (1°) and 2° infections by sorting single B cells from peripheral blood and generating recombinant monoclonal Abs (rmAbs) through PCR-based amplification of V(D)J gene rearrangements of Immunoglobulin (Ig) heavy (H) and light (L)-chains. Among the first 8 peripheral blood mononuclear cell (PBMC) samples sorted from 2° dengue cases, we obtained ~3 plates of single PC-sorted cells (range 1-9 plates/sample), with a mean of 15-20 Ig V<sub>H</sub> and V<sub>L</sub> pairs/plate. Data from the first patient samples analyzed indicated that 47% of recombinant antibodies were DENV-specific, as measured by ELISA to virions of the 4 DENV serotypes. Purified recombinant monoclonal antibodies are being produced and tested for their binding, neutralization and avidity capacities to the four DENV serotypes to generate a map of the frequencies, serotype-, antigen-, and epitope-specificity and functional profile. To this end, we have established a system using surface plasmon resonance (SPR) and purified DENV virions to measure avidity of recombinant mAbs and patient serum. The specificities of the rmAbs will then be correlated with immune status, overall DENV-specific neutralization and serum avidity. The potential impacts of these studies include increased understanding of the immune response to DENV infection, identification of potential therapeutic mAbs, and improved design of dengue vaccines.

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#### HIGHER SOCIO-ECONOMIC STATUS IS PROTECTIVE AGAINST DENGUE VIRUS INFECTION

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Dengue is a mosquito-borne illness that is a major public health problem in developing countries worldwide, particularly in urban settings. The Pediatric Dengue Cohort Study was established in 2004 to study the clinical spectrum and transmission of dengue in children in Managua, Nicaragua. In the first 6 years, annual participation ranged from 3,693 to 3,953 children aged 2 to 14 years. Children were enrolled prospectively, and data was systematically recorded on all medical visits. Participants are encouraged to come in at the first sign of illness and all medical

care is provided free through the study. Those with suspected dengue or undifferentiated fever are tested for dengue by RT-PCR, virus isolation, and serological assays. Additionally, yearly blood samples are collected and tested to determine the incidence of inapparent dengue virus infection. At enrollment, a basic demographic questionnaire was collected which included a number of socioeconomic indicators, and beginning in 2008 a more extensive socioeconomic status (SES) questionnaire was administered on a yearly basis. To examine the relationship between poverty and DENV infection and cases, univariate and multivariable generalized estimating equations (GEE) with a Poisson model were used. First principal component analysis was used to create a wealth index, and the index was then classified into tertiles (very poor, poor, and mid-SES). Variables included in the wealth index were: number of sleeping rooms, crowding, type of floor (dirt, concrete, ceramic/tile), type of housing construction (wood/plastic, concrete), access to water (inside house, outside house), and ownership of durable assets (cars, motorcycles, fans, televisions, refrigerators). Age was included in all multivariable models. In multivariable models, higher SES was a significant protective factor for DENV infection, with an incidence rate ratio (IRR) of 0.89 (95% CI 0.80, 0.98) for poor children when compared to very poor children and an IRR of 0.85 (95% CI 0.76, 0.96) for mid-SES children when compared to very poor children. Likewise, higher SES showed a trend towards being a protective factor for symptomatic disease (IRR 0.89; 95% CI 0.69, 1.14 for poor compared to very poor children and IRR 0.94, 95% CI 0.72, 1.24 for mid-SES compared to very poor children); however the results were not significant. These results support the concept that lower SES conditions are a risk factor for DENV infection.

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### ECONOMIC COST AND DISEASE BURDEN OF DENGUE IN MEXICO: ADJUSTING FOR UNDER-REPORTING

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Despite eradication in the 1970s, epidemic dengue now affects 23 of 31 Mexican states, indicating a need to quantify the burden and cost of dengue to plan and assess control measures. In Mexico, dengue is reportable and case definitions have been promulgated. Protocols for laboratory confirmation include NS1, IgM and IgG tests at state and national levels. In 2011, 70,028 probable dengue cases and 50 dengue deaths were reported; however, official statistics often under-estimate the burden. Mexico, like 89 of 100 endemic countries, lacks cohort studies to ascertain under-reporting directly. Previous studies in the Americas have determined expansion factors (EFs) to estimate the true number of cases as multiples of the reported numbers. These EFs average 2.3 for hospitalized cases and 15.0 for ambulatory cases. We report here a study to quantify dengue burden and cost in Mexico. We are estimating the total number of cases in the public health system by 1) comparing medical records from selected hospitals with Ministry of Health surveillance data, 2) comparing case reports between 201 dengue monitoring facilities and other public facilities, and 3) using laboratory data on numbers of tests distributed, tests performed and positive cases (29.0% of 56,613 samples tested in 2011). We will extrapolate public sector data to the private health sector, initially by assuming a number of cases proportional to the relative sizes of the sectors. For example, the private sector has 35,000 beds and 14,000 doctors' offices, while the public sector has 79,000

hospital beds and 64,000 doctors' offices, giving ratios of 0.44 and 0.22, respectively. In addition to EFs, we will estimate 1) direct costs per dengue episode (adjusted by facility type), 2) indirect costs per dengue episode and due to death, and 3) disability-adjusted life-years burden based on literature review with WHO methodology. These procedures can improve estimates of dengue burden and cost both in Mexico and other countries in the region.

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### DENGUE VIRUS PRM/E STRUCTURES REQUIRED FOR BUDDING AND INFECTION

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While the functions of Dengue virus (DENV) prM/E are well studied, most functional structures of the protein have only been partially mapped. Using Shotgun Mutagenesis technology, a comprehensive plasmid mutation library for DENV-3 prM/E was created in which each prM/E residue was individually mutated to a defined substitution, expressed in human cells, and analyzed for its effect on both viral budding and infectivity. To monitor budding, we used a virus particle production assay in which each prM/E mutant is expressed in a human cell line expressing the nonstructural genes required to produce DENV virus particles that can be detected in the media. To monitor infectivity, we used an infection assay in which each prM/E mutant is incorporated into infectious DENV reporter virus particles (RVPs) carrying a luciferase reporter that were used to infect permissive target cells. We also tested the contribution of each mutation to prM/E folding by measuring the reactivity of each mutant with conformation-dependent antibodies. In total, >1,100 mutants of DENV prM/E were individually tested. Critical residues comprising structures that contribute to each function were mapped onto the crystal structures for prM and Env in order to visualize and better understand how it functions.

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### USE OF COMMERCIAL ELISA KITS IN DETERMINING THE PRESENCE OF DENGUE VIRUS NONSTRUCTURAL PROTEIN (NS1), ANTI-DENGUE IGG AND ANTI-DENGUE IGM IN HUMAN SERUM SAMPLES SUPPORTING DENGUE VACCINE DEVELOPMENT

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The CYD dengue vaccine is currently in phase III efficacy trials. Dengue cases in these trials are assessed by many measures including commercial kits for NS1 protein (BioRad) and anti-dengue IgM and IgG (Focus Diagnostics). The NS1 Ag ELISA is part of an algorithm to identify virologically confirmed dengue cases, the IgM and IgG ELISAs are used to serologically characterize dengue infections in an efficacy trial setting. The performance of these commercial kits in a clinical testing laboratory (CVD Mahidol) was assessed by determination of sensitivity, specificity, and serostatus agreement with an independent laboratory (Focus Diagnostics). Three independent assay runs for each commercial kit were executed, using sample panels of known NS1, anti-dengue IgG or anti dengue IgM serostatus. Of the results, 100% (90/90) of the NS1, 88.8% (80/90) of the anti-dengue IgG ELISA, and 98.8% (89/90) anti-dengue IgM results were correctly classified as positive or negative. The resulting sensitivity and specificity were both 100% for the NS1 ELISA, sensitivity was 93.3% and specificity was 84.4% for the anti-dengue IgG ELISA, and sensitivity was 100% and specificity was 97.8% for the anti-dengue IgM ELISA. The sensitivity and specificity determinations for the testing laboratory were within 10% of the independent laboratory for each of the three ELISAs indicating no evidence of a difference between laboratories. From these

data we conclude that these assays perform equivalently between the testing laboratory and an independent laboratory, and that specificity and sensitivity are acceptable for NS1 and anti-dengue IgM. The specificity of the anti-dengue IgG ELISA suggests that test data close to the threshold must be interpreted cautiously, as normal assay variability can result in identification of these samples as positive or negative. During efficacy trials, samples may be tested using all three commercial kits, these analytical tools will be informative for dengue case characterization.

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#### CLINICAL RULE TO PREDICT RECURRENT SHOCK IN DENGUE SHOCK SYNDROME

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Dengue has become a major public-health burden in tropical and subtropical areas of the world, mostly in South-East Asia and Western Pacific Regions. This disease ranges from asymptomatic or mild fever (DF) to severe dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Mortality in dengue infection is mostly due to the severe form of dengue infection especially the DSS. However, very few study described the recurrent shock that reportedly affects the protocol therapy. Here we aim to find the prevalence of recurrent shock and develop a prediction rule to identify these cases in the early stage of DSS. A prospective study was conducted in two hospitals in Vietnam to identify specific predictors of recurrent shock. Independent predictors were identified using uni-variate analysis. The data set was randomly split into two data sets including derivation and validation sets. The binary logistic regression was used to derive a scoring system for identification of recurrent shock using the derivation set. The performance of the prediction rule was evaluated by receiver operating characteristic (ROC) curve analysis of validation set. Our study included 444 DSS patients, 126 of whom had recurrent shock (28%). The uni-variate analysis showed that shorter admission day, subcutaneous bleeding, gastrointestinal bleeding, ascites, hemoconcentration, low platelet count, narrow pulse pressure were risk factors for development of recurrent shock. A prediction rule was developed based on an equation of five variables including admission day, subcutaneous bleeding, ascites, blood platelet count, blood leukocytes, and pulse pressure. Our rule showed a relative high sensitivity at 68% and a moderate specificity at 59%, with area under the curve at 0.701. In conclusion, we have derived a clinical rule that could assist clinicians to predict the recurrent shock in the early stage of DSS that could assist clinicians to closely monitor the critical patients.

### 638

#### OBSERVATIONS FROM TWO COMMUNITY LYMPHOEDEMA INITIATIVES IN INDIA AND ETHIOPIA

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Lymphoedema or elephantiasis is a common and devastating public health problem in many developing countries. Epidemiologically significant causes include infection for example filariasis or due to geodermatological interactions in the case of podoconiosis. Detailed examination by dermatological specialists have not been reported previously to assess

the specific impact on the skin. Observation of patients attending lymphoedema community clinics in India and Ethiopia were undertaken in November 2010. Patients were examined by dermatologists in both settings and diagnoses recorded. Whilst in both settings the majority of cases were deemed clinically consistent with the main local cause, other causes of leg swelling and additional dermatoses were identified. This presentation details the findings of the diagnoses observed together with a brief description of the two community services in place for caring for this condition from which many millions of those living in resource poor settings remain untreated and uncared for. Lymphatic failure regardless of the underlying cause produces similar clinical features. Whatever the underlying cause for lymphoedema simple skin care intervention can have a dramatic effect on clinical appearance and severity of the disease as well as quality of life.

### 639

#### FILARIAL POLYPARASITISM COMPLICATES EFFORTS TO ELIMINATE LYMPHATIC FILARIASIS IN CENTRAL AFRICA

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The Global Programme to Eliminate Lymphatic Filariasis aims to eliminate the infection in all 73 endemic countries by 2020. Most countries outside of Africa have completed mapping and mass drug administration (MDA) is underway or even completed. In contrast, many countries in Africa have not yet started MDA and mapping data are often inaccurate or incomplete. Accurate, fine-grained mapping is especially important in Central Africa, because ivermectin used in MDA sometimes causes serious adverse events in persons with heavy *Loa loa* infections. Although the Democratic Republic of Congo (DRC) is believed to have the highest LF burden in Central Africa, implementation of MDA for LF has been delayed because of inadequate mapping of LF and because of coendemic loiasis. The aim of this study was to assess the prevalence of LF in Mambasa territory in northeastern DRC prior to implementation of MDA. Microfilaria testing was performed with 60 µL night blood smears, and *Wuchereria bancrofti* antigenemia was detected by the ICT card test. 1,226 subjects (>5 ys) were tested in 14 villages. Slides containing large sheathed microfilariae (Mf) were sent to WUSM for speciation of Mf by microscopy and by qPCR. Prevalence rates for *Mansonella perstans* (with thin, unshathed Mf) ranged from 44 to 98%. Prevalence rates for *W. bancrofti* antigenemia ranged from 0 to 10% and exceeded 1% in 13 villages. Prevalence rates for large, sheathed Mf (either *L. loa* or *W. bancrofti*) ranged from 10-26%. Some samples had > 20,000 large Mf/ml, and 14% of those with large Mf had > 2,000 large Mf/ml. It is very difficult to verify the absence of *W. bancrofti* Mf by microscopy in thick smears that also contain high numbers of *L. loa* Mf. qPCR testing showed that 183 of the 184 blood samples with large Mf were positive for *L. loa*; 18 of these slides contained *W. bancrofti*. One slide contained Mf of *W. bancrofti* and *M. perstans* without *L. loa*. Since *L. loa* Mf have diurnal periodicity, it is likely that loiasis prevalence rates and infection intensities in the study area are much higher than those detected in night blood in our study. Since *W. bancrofti* antigenemia and Mf rates exceed 1%, Mambasa territory qualifies for MDA, although it may not be safe to use ivermectin in this area. This study illustrates difficulties associated with mapping and implementing MDA for LF in Central Africa.

## 640

**HEMOPARASITES IN CAPTIVE NON-HUMAN PRIMATES: RISKS FOR PUBLIC AND ANIMAL HEALTH**

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Poaching and trade of wildlife for local and international exotic pet markets result in frequent close contact between human and non-human primates (NHP). This forced sympatry, coupled with the phylogenetic proximity of humans and NHPs, provides multiple new opportunities for disease emergence. Limited information is available about the presence of bloodborne parasites affecting NHPs in Latin America. Isolated reports have been published but there are no systematic, comprehensive and large scale studies. We conducted a prospective study to estimate the prevalence of blood-borne parasites in captive NHPs that could pose a potential risk for humans. NHPs were sampled in all major cities in the Amazon Basin (Yurimaguas, Iquitos, Pucallpa and Puerto Maldonado) and at two cities with major trade (Lima and Cuzco). All species found were studied, aiming at reaching at least 30 specimens per the three major genera, woolly (*Lagothrix* spp.), capuchin (*Cebus* spp.), and squirrel (*Saimiri* spp.) monkeys, most commonly found in captivity in Peru. Peripheral blood samples (1-3ml) from the femoral vein were collected from chemically restrained primates following verbal consent from their owners. Knott's concentration method and blood smears were performed. Slides were assessed by microscopy at NAMRU-6 by trained staff under the supervision of senior microscopists. Currently, 60 monkeys from zoos and wildlife rescue centers, 41 from wetmarkets and 34 household pet monkeys have been sampled. Forty-nine squirrel, 19 woolly, 33 capuchin, and 34 monkeys belonging to other species have been included. The frequency of microfilaremia was 55% for squirrel monkeys and between 0-33% in the other species. Mixed infections, including *Dipetalonema* spp. and *Mansonella* spp., were found in ten squirrel monkeys. Furthermore, trypanosomiasis was detected in *Saimiri sciureus* (4/25), *Lagothrix lagotricha* (1/9) and *Cebus apella* (1/7). Also, a *Plasmodium* spp. parasite has been found in one squirrel monkey. Wild-caught squirrel monkeys, confiscated from the pet trade, were positive for the three types of hemoparasites reported here, highlighting the risk of introducing these animals to urban settings through the pet trade. Our findings suggest that bloodborne parasites are highly prevalent among Peruvian NHPs and may pose a serious risk to public and animal health that has been largely understudied.

## 641

**LYMPHATIC FILARIASIS TRANSMISSION IN 3 ECO-CLIMATIC AREAS AND THE INNER DELTA OF NIGER RIVER IN MALI**

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Lymphatic filariasis (LF) is a public health problem because of its serious sequel including elephantiasis and hydrocele. Given the endemicity of LF in all regions of Mali, the potential variations of the endemicity level according to the eco-climatic areas and the need of a better use of scarce resources, a parasitological and entomological study has been conducted in 3 eco-climatic areas and the inner delta of Niger River in Mali. A cross-sectional study was undertaken for the parasitological aspect and consisted to make 3 slides of night thick smear of 20  $\mu$ l of blood each. Volunteers' brief medical examination for LF disabilities (hydrocele and elephantiasis) was done at the beginning. For entomological assessment

of LF transmission was done through mosquito collection using Human Landing catch (HLC) in August, October and December 2010 in two villages of each of the three eco-climatic areas over the five existing in Mali: southern Sudan, northern Sudan, and Sahelian areas and one village in the Niger river inner delta (Rice growing area). The collected vectors infection rate was determined using a PCR on pools of 20 mosquitoes. Collected mosquitoes (*Anopheles gambiae* s.l and *An. funestus*) were stored in pools of 20 before the PCR assay aimed at detecting Wb DNA and infection rate estimation using the Poolscreen 2 software. Of the 1,017 subjects tested in the 4 ecoclimatic areas, the wb mf prevalence was 16% in the southern Sudan area, (15.4%) in the northern Sudan area and 0% in the Sahelian and Niger inner delta areas. The prevalence of Hydrocele was 5.81 % in north Sudan area, 1.35 % in south Sudan area and 0% in the Sahelian and the Niger inner delta areas. The prevalence of elephantiasis was 3.5% in southern Sudan area, 0.3% in the Sahelian area, 0.4 in the northern Sudan area and 0% in the Niger inner delta areas. Over the 888 pools of mosquitoes processed by PCR, the average infection rates with 95% confidence interval were 2% (1.2-4.9), 0.4% (0.2-0.9), 0.2% (0.1-0.4), 0.2% (0.1-0.4) respectively in the southern Sudan, the northern Sudan, the Niger inner delta and the Sahelian areas. The north and south Sudan areas are the most endemic for LF and should attract the attention of the decision makers in the context of LF elimination in term of resources allowance.

## 642

**URBAN RISK MAPPING OF LYMPHATIC FILARIASIS IN DAR ES SALAAM, TANZANIA**

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Little is known about the magnitude and distribution of lymphatic filariasis (LF) in coastal urban areas of East Africa, where the disease is caused by *Wuchereria bancrofti* and primarily transmitted by *Culex quinquefasciatus*. This study aimed to map LF prevalence across Dar es Salaam, Tanzania, and determine the local knowledge of disease, potential risk factors and activities of the National LF Elimination Programme in preparation for a large scale-up of mass drug-administration (MDA). A human infection survey and household semi-structured questionnaire were carried out in 10 urban and peri-urban locations (wards) across the three municipalities of the city. LF prevalence of Circulating Filarial Antigen (CFA) was determined using Immunochromatographic test (ICT), and microfilaria (Mf) counts were examined in ICT positive individuals. Evidence of clinical disease was also recorded. In total, 1591 individuals from 259 households were tested for LF infection and 8.5% (n=141) were found to be ICT positive (all Mf=0). Prevalence varied between locations ranging from 2.2% in Buguruni (sub-ward Madenge=0%) to 14.0% in Bunju (sub-ward Bunju A=18.1%). More males (n=85) than females (n=56) tested ICT positive, and individuals < 35 years (61.7%) were the most affected age-group. In total, only 3 cases of lymphodema and 5 hydroceles were recorded. The household questionnaire indicated that 63.2% of those interviewed had heard of LF, but 58.9% did not know the main symptoms, and 61.3% did not know the cause. Approximately half (51.2%) knew about LF Elimination Programme, and one third (35.2%) knew about MDA, with health workers and community members being the most common source of information (>50%). The results and risk maps developed from this study will help the Neglected Tropical Disease (NTD) Programme to implement MDA with special emphasis on advocacy and sensitization of the communities on the disease and the LF Programme itself.

## 643

### FROM CONTROL TO ELIMINATION OF AFRICAN ONCHOCERCIASIS: SHOULD THE FREQUENCY OF IVERMECTIN MASS TREATMENT BE INCREASED?

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The African Programme for Onchocerciasis Control (APOC) has implemented annual mass treatment in areas of African where onchocerciasis is mesoendemic or hyperendemic, aiming to reduce transmission and prevent morbidity. In view of the recent shift in focus from morbidity control to elimination of African onchocerciasis where possible, it has been suggested that APOC should increase the frequency of mass treatment from annual to semiannual, to speed up elimination. To assess under which circumstances this could be worthwhile, we simulated the outcome of annual and semiannual mass treatment in terms of the number of treatment rounds, calendar time and amount of drugs required for elimination. Simulations were done for different settings pertaining to pre-control infection levels and history of mass treatment (number of rounds and population coverage). Simulations were performed in ONCHOSIM, an established microsimulation model for onchocerciasis transmission and control. We updated assumptions about ivermectin efficacy in ONCHOSIM, based on a systematic review of literature regarding the effects of repeated treatments with ivermectin on onchocerciasis, and in particular, adult *Onchocerca volvulus* worms. ONCHOSIM predicted that semiannual treatment (vs. annual) would reduce time required until elimination, especially in foci where mass treatment was started only recently (time reduction of up to 50%). This benefit was lower for foci with high pre-control prevalence of infection or a history of high mass treatment coverage, and foci where coverage of semiannual mass treatment turns out lower than expected. Furthermore, the semiannual treatment strategy required up to 5 more treatment rounds until elimination than the annual treatment strategy. In conclusion, increasing frequency of ivermectin mass treatment in APOC areas may have considerable benefits, depending on several factors. These benefits will have to be weighed against investments and possible logistical barriers for increasing the frequency of mass treatment.

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### HOUSEHOLD-LEVEL RISK MAPPING OF LYMPHATIC FILARIASIS IN URBAN AND PERI-URBAN AREAS OF MOMBASA, KENYA

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Lymphatic filariasis (LF) is endemic in the coastal areas of Kenya where it is caused by *Wuchereria bancrofti*, and transmitted by *Anopheles* and *Culex* mosquitoes. The National LF Elimination Programme is implementing mass drug administration (MDA) using albendazole and diethylcarbamazine citrate (DEC) to interrupt the transmission, however, it is not known if this needs to be extended to the city of Mombasa because of lack of data on the disease. The aim of this study was to map the prevalence of LF across urban and peri-urban areas of Mombasa, and determine the local knowledge of disease and the LF Programme. A human infection survey and household semi-structured questionnaire were carried out in 5 areas of the city. LF prevalence was determined using Immunochromatographic test (ICT), and microfilaria (Mf) counts in ICT positive individuals. Evidence of clinical disease was also recorded. All data were mapped to household

level using geographical coordinates collected during the survey. In total, 510 individuals from 129 households were tested and 2.4% (n=16) were found to be ICT positive. MF counts ranged from 0 to 8. More females (n=10) than males (n=6) tested ICT positive, and ages ranged from 8 to 68. Households with ICT positives tended to be clustered within each area. In total, 5 cases of lymphodema and 6 hydroceles were reported. The household questionnaire indicated that 83.6% of those interviewed had heard of LF, but 57% did not know the main symptoms, and 47.7% did not know the cause. Approximately one third knew about the LF Elimination Programme (30.5%), and about MDA (33.9%), with TV being the most cited source of information (>20%). These results indicate a low sporadic level of LF endemicity in Mombasa and a challenge for the LF Elimination Programme if they were to implement MDA with high coverage.

## 645

### SECONDARY MAPPING OF LYMPHATIC FILARIASIS IN HAITI- DEFINITION OF TRANSMISSION FOCI IN LOW-PREVALENCE SETTINGS

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To eliminate Lymphatic filariasis (LF) as a public health problem, the World Health Organization (WHO) recommends that any area with infection prevalence greater than or equal to 1% (denoted by presence of microfilaremia or antigenemia) should receive mass drug administration (MDA) of antifilarial drugs for at least five consecutive rounds. Areas of low-antigen prevalence (< 1%) are thought to pose little risk for continued transmission of LF. Five low-antigen prevalence communes in Haiti, characterized as part of a national survey, were further assessed for transmission in this study. An initial evaluation of schoolchildren was performed in each commune to identify antigen-positive children who served as index cases for subsequent community surveys conducted among households neighboring the index cases. Global Positioning System (GPS) coordinates and immunochromatographic tests for filarial antigenemia were collected on approximately 1,600 persons of all ages in the five communes. The relationship between antigen-positive cases in the community and index cases was evaluated using multivariate regression techniques and analyses of spatial clustering. Community surveys demonstrated higher antigen prevalence in three of the five communes than was observed in the original mapping survey; autochthonous cases were found in the same three communes. Regression techniques identified a significantly increased likelihood of being antigen-positive when living within 20 meters of index cases when controlling for age, gender, and commune. Spatial clustering of antigen-positive cases was observed in some, but not all communes. Our results suggest that localized transmission was present even in low-prevalence settings and suggest that better surveillance methods may be needed to detect microfoci of LF transmission.

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### LYMPHATIC FILARIASIS ELIMINATION IN NIGERIA: RESULTS OF THE TREATMENT ASSESSMENT SURVEY (TAS)

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The current strategy for interrupting transmission of lymphatic filariasis (LF) in Africa is annual mass drug administration (MDA) with ivermectin and albendazole for 6 or more years. In Plateau and Nassarawa states in north-central Nigeria, MDA was started in 2000 and reached full

coverage in all 30 local government areas (districts) by 2003. In 2008, after 6 rounds of MDA, a large (>30,000) two-state survey assessing district level antigenemia showed that transmission had been interrupted in 10 of the 30 districts, but MDA needed to continue in the remaining districts. By early 2011, long lasting bed nets (LLIN) had been distributed at household level in the two states by the malaria program. In 2012, new WHO guidelines for stopping LF MDA were issued. They called for cost savings by conducting a 'TAS' in grouped 'evaluation units' where districts of similar epidemiological characteristics are joined. We grouped the remaining 20 MDA districts into four EUs (two in each state) based on the 2009 survey results. Districts with an overall 2008 antigenemia of  $\leq 2\%$  were joined (called Group 1) and district  $>2\%$  antigenemia were joined (Group 2). Our assumption was that Group 1 EUs were most likely to 'pass the TAS' and move on to stop MDA and enter into post MDA surveillance. A cluster survey was conducted of primary-school children (6-7 years of age) based on an algorithm provided in the TAS manual that called for a minimum sample size of 1692 per EU with a critical cut off point of 20 positives per EU resulting in failure for that EU. A total of 7,131 children were tested in 173 schools: 43 schools per EU. Group 1 in both Plateau and Nassarawa passed with 8 and 3 ICT positives respectively, and are able to stop MDA. Group 2 also passed in both states with only 10 and 3 positives. Challenges faced that will be discussed included civil unrest and government labor strikes.

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### INTERRUPTION OF ONCHOCERCIASIS TRANSMISSION IN THE ABU HAMED FOCUS, NORTHERN SUDAN

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The isolated Abu Hamed focus in Northern Sudan represents the northernmost endemic area of onchocerciasis in the world with 118,034 individuals at risk. Communities in the focus have undergone annual community-based treatment with Ivermectin (CDTI) with variable coverage rates since 1998. The CDTI control program has been upgraded to biannual treatments in 2006 with baseline entomological, parasitological and serological data collected in 2007. Follow up assessments of biannual treatments and CDTI were performed in 2011. *Onchocerca volvulus* O-150 Pool screen analysis showed no parasite DNA in 16,489 *Simulium damnosum* flies collected from Dec 2010 to Nov 2011 (95% confidence interval upper limit (UL) = 0.042 per 2000 flies) compared to 0.168 (0.0.099-0.376 95% CI) in 2007. Screening for skin snips and onchodermatitis in 536 individuals in 8 villages in and around the focus revealed no microfilariae or skin disease (95% CI UL = 0.0075%) in contrast to mf rate of 0.45% in 2007. Similarly, sera collected from 6,756 school children  $\leq 10$  years old showed no evidence of *O. volvulus* Ov16 IgG4 antibodies (95% CI UL = 0.0007%) compared to zero (0-1.36% 95% CI) prevalence of the antibodies in the same age group in 2007. Our 2011 assessment of the Abu Hamed focus has met the criteria for interrupted transmission of the disease in the area. CDTI activities were halted in 2012 and post-treatment surveillance (PTS) period of three year was initiated. Challenges to the elimination program and plans for PTS will be discussed.

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### PREVENTION OF TYPE 1 DIABETES BY *LITOMOSOIDES SIGMODONTIS* INVOLVES MULTIPLE MECHANISMS

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Prevention of Type 1 diabetes (T1D) is a major focus of research in our laboratory. We use the rodent filarial pathogen *Litomosoides sigmodontis* (LS) to understand protective mechanisms against T1D. We have shown that LS infection prevents T1D and transfer of splenocytes from infected NOD mice into NOD/scid mice fails to induce T1D. We hypothesized that this results from altered T-cell populations. Flow cytometric analysis of uninfected and infected spleens showed no difference in total frequencies of naïve or memory CD4+ T-cells (41/20% vs. 43/20%,  $p=0.9/0.6$ ) or autoantigen BDC2.5-specific naïve and memory CD4+ T-cells (0.01/0.01% vs. 0.007/0.01%,  $p=0.3/0.9$ ), suggesting that LS may alter function rather than number of autoimmune cells. Helminth secreted products are known immunomodulators. We recently showed that LS protection against T1D depends on TGF $\beta$ . Therefore, we evaluated whether LS excretory/secretory products (ESP) signal through the mammalian TGF $\beta$  receptor. *In vitro* assays using a TGF $\beta$  responsive cell line show that ESP from microfilariae, L3s, male adult, and female adult worms ligate the TGF $\beta$  receptor. Additionally, four weekly injections of NOD mice with male or female ESP delays onset of T1D. The delay is greater in female than male ESP-treated mice (4 v. 2 weeks). Studies are ongoing to assess the functionality of autoimmune CD4+ T-cells during infection and to further evaluate the role of ESP in helminth-mediated protection against autoimmunity.

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### VACCINATION AGAINST *BRUGIA MALAYI* CYSTEINE PROTEASE INHIBITOR(S) (BM-CPI) ALTERS THE *B. MALAYI* MIGRATORY PATTERN IN MONGOLIAN GERBILS

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Cysteine protease inhibitors are reversible, tightly binding inhibitors of cysteine proteases and are secreted by the filarial worms and have been shown to exhibit immunomodulatory properties and function in protective immunity. In human filarial parasite, *Brugia malayi*, 2 cysteine protease inhibitor genes, Bm-CPI-1 and Bm-CPI-2 have been described and Bm-CPI-2 has been shown to modulate the host immune response by inhibiting the multiple cysteine protease activities found in the endosomes/lysosomes of human B lymphocyte lines. In *Onchocerca volvulus* and *Litomosoides sigmodontis* animal models they were shown to elicit protection against infection with L3 larvae. Interestingly, excretory-secretory proteins (ES) of filarial nematodes have been known to also play an important role in immunomodulation and immune protection. For example, in the *B. pahangi* - Mongolian gerbil model system, immunization with ES resulted in a modified pattern of migration of L3s in comparison to the controls. In the ES immunized gerbils, more L3s were isolated at the site of infection compared to adjuvant control, suggesting that anti-ES immune responses limit larval migration. As reported previously. As both Bm-CPI-1 and Bm-CPI-2 are present in ES of *B. malayi*, we hypothesized that they could also play a role in protective immunity to *B. malayi* infection in gerbils, as judged by worm development and distribution in vaccinated gerbils. *E. coli* expressed recombinant Bm-CPI-1 and Bm-CPI-2 were used to immunize gerbils using alum as the adjuvant. Gerbils were challenged with L3s and parasites were recovered on day 42 post-infection. There was no significant reduction in worms in Bm-CPI-1 and Bm-CPI-2 immunized gerbils in comparison to alum only controls. However, both Bm-CPI-1 and Bm-CPI-2 vaccinated groups had more worms in the heart and lungs

than control gerbils and fewer worms were found in the lymphatics of the Bm-CPI-1 and Bm-CPI-2 vaccinated groups in comparison to controls. Especially with Bm-CPI-1 vaccination, these changes in distribution of worms were higher and statistically significant. Our results suggest that immunity induced by CPI-1 and-2 may have an effect on migration of worms away from the lymphatics as more worms are observed in heart & lungs of Bm-CPI-1 and-2 immunized gerbils. This phenomenon in relation to the immunomodulatory function of filarial cysteine protease inhibitors is further discussed.

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### IMMUNOINFORMATICS APPROACH TO VACCINE DESIGN AGAIN LYMPHATIC FILARIASIS

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As the 2020 deadline for the eradication of lymphatic filariasis approaches, reliable prophylactic measures to fight against recrudescence and/or drug resistance may be in critical need. Currently, mass drug administration (MDA) rounds have been shown to be effective at reducing parasite burden and overall prevalence within participating endemic countries. Within such countries, MDA stopping points are being considered based on careful algorithms such as those used during transmission assessment surveys (TAS). However, TAS endpoint predictions have not been verified by any prior field validations. Therefore, recrudescence becomes an issue of concern. Therefore, the development of a vaccine could prove to be a more reliable means of prophylaxis. Furthermore, existing gene to vaccine immunoinformatics tools could be used to expedite the selection of highly immunogenic T-cell epitopes for the development of a DNA vaccine. Thus, a large-scale immunoinformatics screening approach was used for *Brugia malayi*, one of the three causative agents for LF, combining GenBank database of 11,465 proteins with EpiVax immunoinformatics software. Of these, 1,774 proteins demonstrated putative immunogenicity. Of these, 456 proteins were predicted to have extraordinary immunogenicity properties. Current studies also indicate homology of the *Brugia malayi* putative immunogenic proteins with *Wuchereria bancrofti* proteins and *Loa loa* proteins.

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### GLYCAEMIC PROFILE ON HIV+ PATIENTS ON HAART IN KIGALI UNIVERSITY TEACHING HOSPITAL: A REVIEW OF 117 CASES

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HAART has been reported to be associated with new-onset diabetes mellitus. However the data available in Rwanda is scarce. This study aimed to determine the prevalence of diabetes mellitus in HIV-infected patients receiving ART. In this prospective study, HIV-infected patients on HAART who attended the clinic of Kigali University Teaching Hospital from February to August 2011 were studied. Fasting plasma glucose was performed in each patient. There were 117 patients with a mean age of 43.5 (range, 15 to 65) years and male were 46.2%. The most common risk factor was an habitual physical inactivity, retrieved in 42 patients, followed by an overweight status (BMI $\geq$ 25 kg/m<sup>2</sup>) for 36 patients (30.8%). The waist-to-hip ratio was abnormal in 16.7% of male patients and 70.3% of female patients of this study. 29 patients (24.8%) presented a lipodystrophy syndrome. Changes of glucose homeostasis have been observed in 48 patients (41%), 18 of them having diabetes mellitus (15.4%). No factor (either traditional risk factors or HAART regimen characteristics) appeared to have influenced the occurrence of impaired fasting glucose among the patients of this study. Glucose impairments are fairly high among HIV-infected patients receiving HAART, especially in those presenting a

lipodystrophy syndrome. Therefore, those patients should be submitted to a regular Fasting Plasma Glucoses. However, a large-scale study is undoubtedly required to confirm our results.

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### FACTORS INFLUENCING ADHERENCE TO ANTIRETROVIRAL THERAPY IN ADOLESCENTS AT BOTSWANA-BAYLOR CHILDREN'S CLINICAL CENTRE OF EXCELLENCE - A QUALITATIVE STUDY

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The aim of the study was to determine the factors that influence adherence to ART among adolescents who contracted HIV through vertical transmission. Qualitative research using descriptive phenomenology was conducted at Botswana-Baylor Children's Clinical Centre of Excellence. The meta-theoretical assumptions were that the experience of adolescents who are HIV positive and taking ART would put them in a position to give insight into what makes one to adhere or not to adhere to treatment and that the critical truth can be found through in-depth individual semi-structured interviews about the factors that influence adherence to ART. Data was collected using in-depth individual semi-structured interviews. Eight (8) adolescents between 14 and 19 years who had been on ART for minimum of 4 years were interviewed. Thematic analysis of data was done and five (5) themes emerged from the participants' description of the experience of taking ART over a long period of time. The themes that emerged indicated the factors that influence adherence to ART, and they included knowledge and positive beliefs about ART, need for support, ART difficult treatment regimen, having a regular doctor and psychosocial emotional needs. The findings suggested that the adolescents who contracted HIV through vertical transmission require support while continuing on a simplified long-term ART regimen after an assessment of their psychological well beings and periodic checks.

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### PREVALENCE OF MALARIA AMONG HIV SEROPOSITIVE CLINIC ATTENDANTS IN FIVE HOSPITALS IN GHANA

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Malaria is associated with an increase in viral load and fall in CD4-cell count. Conversely, HIV infection also disrupts the acquired immune response to malaria and the efficacy of antimalarial drugs. This study was carried out in five Ghanaian hospitals to estimate the prevalence of malaria among HIV patients and also to assess possible factors that influence the epidemiology of malaria and HIV co-infection. The descriptive cross sectional study reviewed and collected data on malaria, using Case Record Forms from HIV patients' folders in five hospitals in the Brong Ahafo and Ashanti Regions of Ghana. Ethical approvals were obtained from three recognized Ethical Review Committees. The total 933 patients were made of 272 (29.2%) males and 661 (70.8%) females. Majority were between 21- 41 (63.6%) years old. In the 933 folders reviewed, 339 (95.5%) of 355 were clinically suspected and referred to the laboratory for confirmation of malaria diagnosis. Only 4.4% (95% CI: 2.2, 6.6) patients tested were confirmed cases of malaria. Fever, was not significantly associated with confirmed malaria (OR=3.11, 95% CI: [0.63, 15.37], P=0.142), however, fever was presumptively used to diagnose

malaria (OR=4.11, 95% CI: [2.83, 5.96], P<0.001). Huge missing data were recorded due to the poor keeping of records at all the sites. In conclusion, a low prevalence of confirmed malaria, 4.4% (95% CI: 2.2, 6.6), was recorded among HIV patients from Ghana. The prevalence could also be attributed to the high cases of malaria diagnosed presumptively (37.0%), (OR=4.11, 95% CI: [2.83, 5.96], P<0.001). Evidence based diagnoses and treatment of malaria should be improved. Demographic characteristics, CD4 count levels and ART status of patients were not significantly associated with malaria. This may be due to the poor records keeping at all sites.

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### EXTENT OF INADEQUATE RECORDS KEEPING AND THE EFFECT ON PATIENT CARE FROM FIVE HIV MANAGEMENT HOSPITALS IN GHANA

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Medical records serve many functions but their primary purpose is to support patient care and to improve in control programmes. The importance of completeness of patients' records cannot be overemphasized, especially for the purpose of auditing, research, and medico-legal reasons. However gross inadequacies are often noted. We carried out this review to observe the quality of records keeping in five HIV management clinics. The study reviewed 933 patients' folders randomly selected systematically from five hospitals in two regions of Ghana. Quality assessment was made on the extent of missing and incomplete records in the folders of patients. Case Record Form was developed to collect data on HIV and malaria from the management clinics. Ethical approvals were given by the Ethical Review Committees of Ghana Health Service, London School of Hygiene and Kintampo Health Research Centre and permissions from the hospitals. Data analyses were done using Stata 11 software. The study revealed extensive amount of missing records in patients' folders across the sites. The extent of missing data ranges from 3.5% on patients' initial CD4 counts to 90.5% of TB screening results and 100% on malaria preventive practices. Incomplete records were also collected on variables such age, weight, fever and treatments given. The quality of record keeping in the HIV management clinics was inadequate. This is comparable to what was observed by Peltzer et al, 2010. It is important not only for patients' care, but also to improve in the existing control measures. Though the case is argued for establishing evidence-based standards for record keeping, where such standards are present, we recommend regular audits and disciplinary measures to make sure health workers adhere to existing protocols for proper documentation. Also, adequate staffing should be provided to handle data collection at the various HIV management clinics in addition to the clinical staff.

## 655

### GENETIC ANALYSIS OF HIV-1 SUBTYPES IN RWANDA

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Human immunodeficiency virus type 1 (HIV-1) infection cases in Rwanda among adults are estimated to be 200,000, and among these more than 50% are on highly active antiretroviral therapy (HAART). The recent emergence of drug resistant HIV strains has created an urgency to evaluate the circulating HIV genotypes. As a result, the National

Reference Laboratory of Rwanda and its collaborating partners have initiated a HIV genotyping study to monitor drug resistance in the infected population. The objective of the study was to determine the HIV subtypes among submitted samples and for which sequences were generated. Quantification of viral load (VL) in plasma samples was performed using Roche COBAS® AmpliPrep/Cobas® and Taqman® RealTime HIV-1 assays. Viral RNA was extracted using Viroseq v2.0 technology (Abbott) and sequencing was accomplished with the ABI 3500xl (Applied Biosystems). A total of 134 randomly selected nucleotide sequences were analyzed in the protease (PR) and reverse transcriptase (RT) regions with the HIVseq program (Stanford University) for classification based on subtype. Sequences for both PR and RT were generated for all samples. Among the 134 sequences analyzed in the PR region, 59 (44%) were circulating recombinant forms (CRF01\_AE), 56 (41.8%) were subtype A, 15 (11.2%) were subtype C, 3 (2.2%) were subtype D, and 1 (0.8%) was subtype K. For the RT region, 18 (13.4%) were CRF01\_AE, 91 (67.9%) were subtype A, 21 (15.7%) were subtype C, 4 (3.0%) were subtype D, and 1 (0.8%) was subtype K. Concordant PR/RT subtypes A, AE, C, D, and K were found in 46 (34%), 11 (8.2%), 15 (11.2%), 3 (2.2%), and 1 (0.8%) respectively. Genetic tree of HIV1 in Rwanda. In conclusion, this is the first study to extensively evaluate the HIV-1 strains in Rwanda in the *pol* gene region and to report on HIV-1 subtype K. Our RT data support observations by others that about 70% of HIV-1 in Rwanda is subtype A. However, our PR data indicate that the prevalence of subtype A and CRF01\_AE is comparable at 44.0% and 41.8% respectively. Overall, more than 80% of HIV-1 circulating in Rwanda are either subtype A or CRF01\_AE in the *pol* region.

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### BAFFLED BY FALLING OR STAGNATED CD4 COUNTS IN YOUR PATIENTS ON ANTIRETROVIRAL THERAPY (ART) DESPITE ADEQUATE HIV VIRAL SUPPRESSION? DO NOT IGNORE BRUCELLOSIS

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Brucellosis is the commonest zoonotic disease in the world being endemic or re-emerging in many areas including Africa. We noticed a significant decline or stagnation in CD4 counts in some of our clients who were adherent on their ART and had no evidence of active pulmonary tuberculosis (PTB). Then we got the landmark case of brucella meningo-encephalitis in AIDS that prompted this case study. She had been on ART for 24 months with an undetectable viral load (<400 copies/ml) but the CD4 counts fell from 565 to 310 over 8 months and developed delirium plus seizures. Serology and cerebrospinal fluid examination identified neurobrucellosis and cyptococcal meningitis as well (despite her counts!). After anti-brucella treatment the counts bounced back to 610 and the neurological features cleared. This suggested that brucellosis was responsible for the phenomenon so we began seeking other cases. Of 1400 patients on ART, 21 had declining/stagnated counts despite good adherence. Fourteen had either brucellosis or urinary TB but detectable viral loads (>400 copies/ml) (excluded), 5 had undetectable viral loads and brucellosis (included in study), 2 had undetectable virus with urinary tuberculosis (excluded). All the 5 included were female with duration on ART of 10 to 42 months. Two had spondyloarthritis, 2 had neurobrucellosis and 1 had enteritis. Four had CD4 drops that ranged between 10 and 45%. One had stagnated at 185 counts despite being on ART for more than 20 months. All got 12 weeks of Doxycycline plus Cotrimoxazole and 2 weeks of Streptomycin and their CD4 counts normalized. Our study shows that brucellosis in AIDS patients on ART, can cause CD4 counts to significantly fall/stagnate despite the HIV virus being adequately suppressed. Patients who have this situation should be screened for brucellosis. Bigger studies on the impact of brucellosis on HIV/AIDS and effectiveness of ART are needed.



### **SURROGATES OF HIV DRUG RESISTANCE MUTATIONS IN PATIENTS FAILING FIRST LINE THERAPY IN JOS, NIGERIA: AN INVALUABLE TOOL IN RESOURCE CONSTRAINT COUNTRIES**

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Nigeria with a population of 160 million people has HIV prevalence of 4.1% accounting for 10% of the world HIV burden. There has been a rapid scale up of antiretroviral therapy in Nigeria from <10,000 in 2002 to over 300,000 patients in 2010. The emergence of antiretroviral drug resistance is now a major challenge to successful treatment. Development of drug resistance in Nigeria like in other developing countries is facilitated by the initial erratic supply of HIV drugs, stigma, poverty, poor adherence, inadequate infrastructure and insufficient human resources. Drug resistance testing is largely unavailable in resource constraints countries. There is therefore the need to determine surrogates that are available in resource constraint countries. We hypothesized that the prevalence antiretroviral drug resistance mutations among patients failing first line antiretroviral drugs in our setting was high and that declining CD4 counts, decreasing weight, HIV viral load  $\geq 4 \log$  copies/ml (at virological failure), duration on antiretroviral therapy >1 year, poor adherence and presence of opportunistic infections (at virological failure) were surrogates of antiretroviral drugs resistance mutations. It was a cross sectional study carried out in Jos, one of the largest HIV treatment site in Nigeria. The study was carried out between July and October, 2010. One hundred consenting patients were randomly selected from 320 patients that had virological failure. Multiple Logistic regression analysis was used to determine factors independently associated with antiretroviral drug resistance. The overall prevalence of antiretroviral drug resistance was 82%. HIV viral load  $\geq 4 \log$  copies/ml (at the time of virological failure) ( $P=0.01$ ), duration on antiretroviral therapy >1 year ( $P=0.03$ ) and presence of opportunistic infections ( $P=0.03$ ) were found to be associated with drug resistance mutations. These may be used in resource poor settings as surrogates of antiretroviral drugs resistance mutations.

### **BARRIERS TO UTILIZATION OF PROVIDER-INITIATED HIV COUNSELING AND TESTING SERVICES AMONG TUBERCULOSIS PATIENTS: A CASE OF RHODES CHEST CLINIC NAIROBI, KENYA**

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Tuberculosis (TB) continues to be one of the most important global public health threats particularly in Sub-Saharan Africa where majority of TB patients are also infected with HIV. HIV prevalence among TB patients in sub-Saharan Africa is 70%. In 2007, WHO recommended that countries with high co-infection rates develop TB and HIV collaborative activities, including provider-initiated HIV testing and counseling (PITC) of TB patients in TB clinical settings. The objective of the study was to determine the barriers to utilization of provider initiated HIV counseling and testing services among TB patients. A cross sectional survey of TB suspects visiting Rhodes chest clinic, Nairobi was conducted. Consenting patients who visited the clinic during October to December 2010 were the study subjects. Data was collected using a standard questionnaire. A chi square test was used to interpret results for each possible barrier in terms of utilized versus declined to utilize

HIV counseling and testing services. Written informed consent was obtained from all participants. Eighty three percent of TB patients tested for HIV infection. The main reasons for not being tested were that they don't trust confidentiality (17.9%), fear of positive test results (11.9%), fear of discrimination (10.4%) and self perception of low risk (7.5%) ( $\chi^2=29.473$ , 9 df,  $p=0.030$ ). Factors that were significantly associated with utilization of PITC services were age ( $\chi^2=11.319$ , 2df,  $p=0.003$ ), gender ( $\chi^2=5.919$ , 1df,  $p=0.015$ ), level of education ( $\chi^2=116.045$ , 2df,  $p=0.0001$ ), HIV stigma ( $\chi^2=36.947$ , 3df,  $p=0.0001$ ), awareness of HIV-TB link ( $\chi^2=22.767$ , 2df,  $p=0.0001$ ) and discussion of HIV/ TB link by nurse ( $\chi^2=59.232$ , 2df,  $p=0.0001$ ). In conclusion, utilization of PITC services by TB patients was high at the Chest clinic. The Kenya National AIDS Control Council, National Leprosy and Tuberculosis Program (NLTP) and TB/HIV Partners should scale up community awareness about HIV-TB co infection and train all providers on collaborative HIV-TB services. Advocacy for HIV screening for all TB patients should also be increased.

### **MONITORING OF SEXUALLY TRANSMITTED INFECTIONS IN SENEGAL: A NATIONAL SURVEY CARRIED OUT IN 2006 AND IN 2010 AMONG RESPECTIVELY 596 AND 570 FEMALE SEX WORKER IN SOME STIS CENTRES OF SENEGAL**

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This work aimed to determine the prevalence of sexually transmitted infections (STI) in the group of the Female Sex Workers (FSW) in Senegal. We have done a behaviour survey combined to a biological investigation. The vaginal specimen collection was done by the FSW herself and blood was collected at the elbow. The vaginal secretion was used for the diagnosis of vaginal candidiasis, *Trichomonas vaginalis* vaginitis and bacterial vaginosis; blood was analyzed for diagnosis of syphilis and the HIV infection. On a total of 1166 women, 596 were tested in 2006 and 570 in 2010. The age varied from 15 to 62 years with an average of 35,09 years in 2006 and 36,59 years in 2010. All were Female Sex Workers registered with in a national medical centre or working as a clandestine. *T. vaginalis* was found in 11, 2% of the cases in 2006 and 14, 9% of the cases in 2010 with a non significant difference whereas the prevalence of the vaginal candidiasis was 7, 6% in 2006 and 11, 1% in 2010. The bacterial vaginosis affected 38,9% in 2006 and this STI has affected more than the half of the FSW in 2010 (52,3%). Bacterial vaginosis was almost always associated with a local inflammatory reaction. The rate of Syphilis went from 12,9% to 4,0% in 2010. The HIV prevalence was 19,9% among the FSW in 2006 and 11,9% in 2010 whereas the pregnant women tested in 2006 gave only a rate of 0,8% (0,6 to 4,3% according to area), thus confirming the HIV concentrated epidemic in Sénégal. Among the FSW, HIV co-infection with bacterial vaginosis were noted in 57% and 61,8% respectively in 2006 and 2010. This work shows the stability of HIV infection in Senegal but also it suggests some future orientations in care, support, prevention and research on STIs. It shows that the microbiological part of the national survey and monitoring of STIs must be conducted in a sentinel manner.

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### IMPACT OF DEWORMING ON HIV AND VACCINE SPECIFIC ANTIBODY RESPONSES AMONG ASCARIS AND HIV CO-INFECTED ADULTS

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In Africa, individuals are frequently co-infected with helminths and HIV. Both infections impact the host immune response through immune suppression and dysregulation. As a result, these infections may also lead to diminished vaccine efficacy in populations where co-infection is prevalent. We determined the impact of deworming on humoral responses to HIV infection and previously administered vaccinations within a nested cohort study. HIV and *Ascaris* co-infected individuals were randomized to 400mg albendazole for 3 days (n=17) or placebo (n=19). Antibody responses to HIV antigens were measured using protein microarray before and 3 months post-treatment. Of the 132 HIV antigens examined, 21 were sero-reactive. Among sero-reactive antigens, there was no difference in average change in signal intensity over 3 months between the treatment groups (all p>0.20). In addition, vaccine antibody responses were measured using commercially available ELISAs to tetanus and measles among dewormed (n=16) and placebo treated (n=19) individuals. All were sero-positive (>0.01 IU/mL) for tetanus IgG at baseline and 3 months. The average change in tetanus IgG response over 3 months was -0.076 IU/mL in the dewormed group and +0.043 IU/mL in the placebo group (p=0.46). At baseline, 69% (n=11/16) of the dewormed group and 79% (n=15/19) of the placebo group were sero-positive for measles IgG. All of the dewormed individuals (n=11/11) compared to 80% of placebo treated individuals (n=12/15) remained sero-positive after 3 months (p=0.25). Additionally, 20% of dewormed (n=1/5) compared to 0% of placebo treated individuals (n=0/4), who were sero-negative at baseline sero-converted to positive after 3 months (p>0.3). Our study lacked sufficient power to detect an immediate effect of deworming on HIV or vaccine responses; however, short or long term impacts may still be important. If treating existing helminth infections modifies host responses to other antigens, greater efforts to empirically deworm in endemic areas may favorably impact responses to immunizations in these regions.

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### HOST IRON STATUS AND IRON SUPPLEMENTATION IMPACT ERYTHROCYTIC STAGE OF *PLASMODIUM FALCIPARUM*

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Iron deficiency is prevalent in children and pregnant women in developing countries, and the World Health Organization recommends routine iron supplementation of children and adults. However, recent evidence suggests that iron deficiency may protect against malaria and that iron supplementation may increase susceptibility to malaria, complicating recommendations for universal supplementation in malaria-endemic regions. The biological mechanisms for both the malaria-protective effect of iron deficiency and the increased risk of malaria associated with iron supplementation remain unclear. To investigate the interactions between host iron status, iron supplementation, and the erythrocytic stage of *Plasmodium falciparum*, we used the *in vitro* model of the erythrocyte stage of *P. falciparum* infection to investigate the effect upon *P. falciparum* erythrocyte invasion and intraerythrocytic growth of (i) host iron status and (ii) iron supplementation of the host. This approach has allowed us to systematically study the *in vitro* growth, development and invasion efficiency of *P. falciparum* in red blood cells from iron-replete healthy and individuals with iron deficiency anemia, before and after

iron supplementation. We observed decreased growth of *P. falciparum* in microcytic RBCs from individuals with iron deficiency anemia as compared to RBCs from iron-replete individuals. We found that *P. falciparum* matured normally through its 48 hour life cycle, but had decreased invasion efficiency into microcytic RBCs from individuals with iron deficiency anemia. When both iron-replete and iron-deficient individuals were given iron supplementation, we observed increased growth of *P. falciparum* in RBCs from individuals with iron deficiency anemia but not in iron replete individuals. It was observed that individuals with iron deficiency anemia receiving iron supplementation had elevated reticulocyte counts, and these reticulocytes had an increased parasite prevalence compared to mature RBCs. Finally, we show that *P. falciparum* is capable of invading and growing within RBCs of all ages, but that *P. falciparum* invasion efficiency and growth steadily decrease with increasing RBC age. These results suggest that host iron status and iron supplementation conspire to mediate host susceptibility to *P. falciparum* infection by altering the dynamics of the RBC population.

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### SATA3 REGULATION OF MMP3 AND CEBPβ IN MALARIA-INDUCED BRAIN DAMAGE

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Our previous results showed that STAT3 is activated by *Plasmodium berghei* ANKA infection *in vivo* and Heme *in vitro*. Heme up-regulates HO-1 and CXCL10 production through STAT3 pathway, and regulates CXCL10 at the transcriptional level *in vitro*. Mutual regulation exists between HO-1 and CXCL10. Since brain endothelial is one of the crucial components of blood brain barrier (BBB), which is severely disrupted and is a characteristic manifestation in cerebral malaria (CM), so we further determine how STAT3 signaling pathway is regulated in the pathogenesis of CM not only using experimental CM model and but *in vitro* human brain microvascular endothelial cells (HBVEC) culture. 1) We analyzed the apoptotic effects of Heme on HBVEC using cell viability and TUNEL assays. 2) We then analyzed Heme-induced JAK/STAT signaling pathway using real time RT<sup>2</sup> Profile PCR arrays. 3) We next examined the effects of STAT3 on MMP3 by introduction of constitutive STAT3 (caSTAT3) and dominant STAT3 (dnSTAT3) into HBVEC. 4) Lastly we measured the expression level of MMP3 in brain tissue in ECM model. Results of these studies demonstrate that 1) Heme causes cell death in HBVEC by increasing apoptosis rate by 20% compared to control. JAK2 inhibition by AG490 protects HBVEC death induced by Heme. During this process, STAT3 is activated indicated by expression of phosphorylated STAT3 (pSTAT3) along with increased levels of MMP3, CXCL10 and HO-1. 2) The results of real time RT<sup>2</sup> Profile PCR arrays are expressed as the fold changes in expression obtained by comparing HBVEC treated with Heme or with vehicle as control. The up-regulated genes (with fold-change greater than 2) include STAT-induced gene matrix metalloproteinase 3 (MMP3), apoptosis-related gene CCAAT/enhancer binding protein (C/EBPβ), Fc fragment of IgG, high affinity Ia, receptor (FCGR1A), Jun B proto-oncogene (JUNB), nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (NFκB), suppressor of cytokine signaling (SOCS3), SOCS4 and signal transducer and activator of transcription 4 (STAT4). The down regulated genes consist of coagulation factor II receptor (F2R) and 2'-5'-oligoadenylate synthetase 1 (OAS1). 3) caSTAT3 up-regulates whereas dnSTAT3 down-regulates MMP3 in HBVEC treated by Heme. 4) MMP3 and CEBP are up-regulated in brain tissues of C57 BL/6 mice infected with *P. berghei* ANKA. In conclusion, MMP3 and CEBPβ which are the two down-regulators of STAT3 pathway might be highly related to malaria-induced damage.

### PRETERM DELIVERY IS ASSOCIATED WITH PREGNANCY MALARIA AMONG WOMEN LIVING IN OUELESSEBOUGOU, MALI

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Over many years of research it has been shown that adult residents of malaria endemic areas have developed immune responses that protect them from severe disease. Women become more susceptible to malaria during pregnancy, especially during the first pregnancy. To investigate the impact of pregnancy malaria on birth outcomes, pregnant women were recruited to a longitudinal study in Ouelessebougou Mali. The women were seen monthly and at any time they were sick by the clinical team. Gestational age was determined by ultrasound. Pregnancy malaria was defined as positive blood smear during pregnancy and placenta blood smear at delivery. The relationship between malaria infection and gestational age was assessed by logistic regression. After excluding twins and still-birth deliveries, the analytical population included 321 mother-infant pairs. Pregnancy malaria was more frequent among first time mothers with more than 50% of the women experiencing at least one malaria episode during pregnancy compared to 28% in multiparous mothers (OR = 3.1 (1.8-5.2),  $p < 0.0001$ ). Similarly, preterm deliveries were more common among malaria-infected primigravidas than multigravidas (34% and 9% respectively). Pregnancy malaria was associated with increased risk of preterm delivery after adjusting for the follow-up time in first time mothers (OR 3.27 (1.01-10.62),  $p = 0.048$ ). In summary, preliminary analysis of a cohort of women living in an area with seasonal malaria transmission suggests that pregnancy malaria is associated with risk for preterm delivery.

### IDENTIFICATION OF A NOVEL ERYTHROCYTE-BINDING LIGAND OF *PLASMODIUM VIVAX* MEROZOITE SURFACE PROTEIN 1 PARALOG, PvMSP1P

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Merozoite surface protein 1 of *Plasmodium vivax* (PvMSP1), a glycosylphosphatidylinositol-anchored protein (GPI-AP), is a malaria vaccine candidate for *P. vivax*. The paralog of PvMSP1, named *P. vivax* merozoite surface protein 1 paralog (PvMSP1P; PlasmoDB PVX\_099975), was recently identified as a GPI-AP. Similar genetic structural characteristics between PvMSP1 and PvMSP1P (e.g., size of open reading frames, two epidermal growth factor-like domains, and GPI-anchor motif in the C-terminus) led us to study this protein. All of the PvMSP1P fragments including 83-, (A, B, and C), 30-, 38-, 42-, 33-, and 19-kDa fragments predicted by the processed structure of PvMSP1 were expressed successfully as recombinant

proteins. We studied the naturally acquired immune response against each fragment of recombinant PvMSP1P and the potential binding ability of each fragment to erythrocytes. The N-terminal (83A) and two C-terminal fragments (33 and 19) reacted strongly with antibody of sera from *P. vivax*-infected patients, producing 50-68% sensitivity and 95-96% specificity, respectively. An *in vitro* cytoadherence assay showed that PvMSP1P, especially the C-terminal 19-kDa region, could bind to erythrocytes. We also found that human sera from populations naturally exposed to *vivax* malaria and antisera obtained by immunization using the recombinant molecule PvMSP1P-19 inhibited *in vitro* binding of human erythrocytes to MSP1P-19. These results provide further evidence that the MSP1P is an essential parasite adhesion molecule in the *P. vivax* merozoite and is a vaccine candidate against *P. vivax*.

### EXPORTED PROTEIN-2 IS ASSOCIATED WITH MEMBRANE-BOUND VESICLES IN THE CYTOSOL OF *PLASMODIUM YOELII* 17X INFECTED RETICULOCYTES

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Malaria parasites replicate inside RBCs and export hundreds of proteins into the host cytosol in order to perform various functions including nutrient import and modification of the host cell cytoskeleton. A subset of these proteins is exported to the host cell surface where they mediate tissue-specific binding of infected RBCs to vascular endothelium. These surface-expressed parasite proteins represent potential vaccine targets as antibodies against them could block parasite sequestration, enhance phagocytosis and/or promote complement mediated lysis of infected RBCs. To identify parasite-encoded proteins exported to the host membrane, we performed a proteomic analysis of membrane proteins in *Plasmodium yoelii* 17X infected reticulocytes which identified eight *Plasmodium yoelii* proteins including the 31 kDa *P. yoelii* Exported protein-2 (PY05892). Full length Exported protein-2 (Exp-2) was cloned from cDNA and expressed as a recombinant protein fused to 6xHis-tag in *E. coli* BL21(DE3) Codon Plus cells. Recombinant Exp-2 was purified from the insoluble fraction by Nickel-NTA chromatography and polyclonal sera was raised against the protein in rabbits and mice. Antibodies against Exp-2 were able to detect the full-length native protein in the membrane fraction of *P. yoelii* 17X infected reticulocytes. Indirect immunofluorescence studies showed that Exp-2 was expressed in all blood stages, localized to the parasitophorous vacuolar membrane (PVM) and was also exported into host cytoplasm on membrane-bound vesicles budding from PVM. Budding of Exp-2 bound vesicles starts as early as ring stages and the number of vesicles increases during the trophozoite and schizont stages. Studies in *Plasmodium falciparum* identified Exp-2 as part of the PTEX translocon machinery present on the PVM where it is involved in export of parasite-derived proteins. However, in our *P. yoelii* rodent model, we show that Exp-2, in addition to PVM localization, is exported into the reticulocyte cytoplasm suggesting a potential role in protein export beyond the parasitophorous vacuolar membrane. Studies are ongoing to delineate the role of Exp-2 in protein trafficking *in vivo* by targeted gene knock out studies and to identify cargo proteins associated with Exp-2 positive vesicles.

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### INACTIVATION OF *PLASMODIUM* AND *BABESIA* PARASITES USING THE S-303 PATHOGEN INACTIVATION SYSTEM FOR RED BLOOD CELLS

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Despite donor screening and testing for specific pathogens, emerging pathogens and organism loads too low to be detected by standard testing methods may result in contamination of red blood cells (RBC) intended for transfusion. A second generation pathogen inactivation (PI) system for RBCs has been developed as a proactive measure to prevent transfusion-transmission of viruses, bacteria and protozoan parasites. This system uses S-303 to crosslink nucleic acids, preventing replication of contaminating pathogens and leukocytes. Glutathione (GSH) is included to quench nonspecific reactions. The system also includes a diluent solution designed to enhance inactivation of pathogens. The aim of this study was to determine the efficacy of inactivation of malaria (*P. falciparum*) and *Babesia* (*B. divergens*) in RBCs by the S-303 PI system. RBCs suspended in AS-5 were prepared from whole blood (450-500 mL) held overnight at ambient temperature and separated without platelet recovery. Four replicate inactivation experiments were performed for each organism. Individual RBC units were used for each replicate. RBC units were inoculated with the organism to be tested and the infected units were then combined with GSH and diluent. A control sample of 5 to 6.6 mL was removed and immediately assayed for viable parasites. S-303 was then added to the RBC mixture resulting in a final concentration of 20mM GSH and 0.2mM S-303. The units were incubated for 3 hours at room temperature before being assayed for residual viable parasites. Preliminary results indicate that *P. falciparum* was inactivated to below the limit of detection and *B. divergens* was inactivated by more than 5 logs. These results indicate that high titers of *Plasmodium* and *Babesia* can be inactivated in RBCs under conditions compatible with blood center operation.

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### DETERMINING THE EFFECTS OF *PLASMODIUM FALCIPARUM* MALARIA ON PRIMARY INFECTION OF B CELLS BY EBV: MODELING EARLY EVENTS IN BURKITT'S LYMPHOMAGENESIS

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Endemic Burkitt's lymphoma (eBL), the most common pediatric cancer in sub-Saharan Africa, is an aggressive B cell lymphoma with two infectious cofactors: Epstein Barr virus (EBV) infection and frequent *Plasmodium falciparum* malaria. Recent research suggests that *P. falciparum* parasites increase the lytic activation of EBV from B cell lines. It was shown that *P. falciparum* parasites exert their effect on EBV through polyclonal activation B cells. *Plasmodium falciparum* parasites contain multiple polyclonal B cell activators, including CpG DNA, which activates toll-like receptor 9 (TLR9), and Pf Erythrocyte Membrane Protein-1 (PfEMP1), which binds surface immunoglobulin as well as CD36. It was recently shown that activation of B cells using CpG oligonucleotide sequences increased the infection and EBV-induced proliferation of B cells. We hypothesized that *P. falciparum* parasites increase the primary EBV infection and EBV-induced proliferation of B cells. Our preliminary *in vivo* studies suggest that *P. falciparum* indeed increases these measures. We observed an increased frequency of EBV infected peripheral blood mononuclear cells in children in a high malaria area compared to those in a nearby low malaria area of western Kenya by quantitative PCR. Our ongoing fieldwork in Kisumu, Kenya, aims to

determine the effect of acute malaria on B cell phenotypes and EBV carriage within the B cell compartment by flow cytometry. Our *in vitro* studies aim to determine the effects of *P. falciparum* on the establishment of EBV latency. We have shown that TLR9 ligation in human PBMC increases surface expression of the EBV receptor, CD21 on B cells. We also showed that TLR9 ligation synergistically increased the proliferation of B cells in culture by CFSE assay. We have expressed recombinant PfEMP1 in order to determine the effect of the other known malaria B cell activator on EBV infection. Overall this work will help elucidate the interaction of *P. falciparum* and EBV with the goal of discovering preventative strategies for eBL.

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### THE GENETIC DIVERSITY OF *PLASMODIUM VIVAX* IN POPULATIONS FROM THE AMERICAS AS INFERRED FROM MITOCHONDRIAL GENOME SEQUENCES

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*Plasmodium vivax* is the most prevalent human malaria parasite in the Americas. Previous studies have contrasted the genetic diversity observed in populations from the Americas to those in Asia and the Pacific, concluding that the populations in the New World exhibit low genetic diversity likely due to their more recent introduction. Here we investigate the genetic diversity of the *P. vivax* population in the Americas and compare the observed pattern with other populations from around the globe. Overall, the Americas as a region harbors a *P. vivax* genetic diversity that is comparable to that observed in Asia and the Pacific. We observe that there is a distinct and relatively ancient clade of *P. vivax* in South America as well as other lineages that could have resulted from independent introductions. Whereas the finding of an ancient lineage is not per se evidence that it was introduced to the region at the time of that lineage's origin, it does provide evidence of a more complex evolutionary history than previously thought. The emerging pattern indicates a strong geographic population structure that could explain previous views that *P. vivax* in this region has low genetic diversity since several studies were carried out in limited geographic areas. We propose that whereas in Asia human migration increases local genetic diversity, the strong geographic structure evident in the Americas has resulted in patterns of low local genetic diversity but high regional diversity. Such a complex pattern needs to be considered in genetic diversity assessments of genes encoding vaccine candidate antigens.

### ASCERTAINING THE RADIATION OF PRIMATE MALARIAS IN ASIA: USING COMPARATIVE APPROACHES TO UNDERSTAND THE EVOLUTION OF MEROZOITE PROTEINS

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There has been great interest in understanding the origin of the different species that cause human malaria. Here we investigate the evolution of major malarial parasite groups that infect non-human primates. We report the result of biodiversity surveys that have allowed us to discover several lineages of primate malarias in Asia, specifically from macaques and orangutans. Overall, we found that the radiations of primate malarias, including those that infect humans, are the result of a complex evolutionary history that includes several host switches. We also found evidence of lineages that could be new *Plasmodium* species in macaques. Then these species are used in comparative approaches focusing on understanding genes important in the invasion of the red blood cell. These non-human primate malarias from orangutan and macaques are used to study antigenic proteins involved in the invasion of the red blood cell (AMA-1 and GPI anchored merozoite proteins). Overall, we found that these proteins are under different forms of natural selection. There are lineage specific selective pressures but also some conserved motifs across species indicating strong purifying selection or functional constraints. We conclude that the study of non-human primate malarias provides valuable information to better understand emerging patterns in the genetic diversity of human parasites.

### LIMITATIONS OF SINGLE GENE MITOCHONDRIAL APPROACHES FOR IDENTIFYING SPECIES OF MALARIAL PARASITES

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Ascertaining the evolutionary history of the protozoan genus *Plasmodium*, which includes the agents of human malarias, has been an issue of great interest in both the malaria and evolutionary biology research communities. Such interest has driven several biodiversity assessments of malarial parasites in natural populations of non-human primates and birds. The use of mitochondrial markers in malarial parasites, specifically partial sequences of the gene encoding Cytochrome B (Cyt B), has been promoted as a method for both species identification and for discovering new species. Here we test both the potential and limitations of mitochondrial genes, including complete Cyt B sequences, as biodiversity assessment tools in malarial parasites by analyzing a group of well-characterized *Plasmodium* species and contrasting the results of single mitochondrial gene approaches with those from complete mitochondrial genomes. We found that CytB has strong phylogenetic information that allows the correct differentiation of distantly related parasites such as the four human malarias. However, CytB cannot reliably uncover many recent phylogenetic relationships of species that radiated at a scale of 2-5 million years ago. Our conclusion is that single gene approaches do not contain enough information to build reliable molecular phylogenies or define new species.

### THE EUKARYOTIC PATHOGEN DATABASE RESOURCE (EUPATHDB): A EUKARYOTIC PATHOGEN GENOME RESOURCE

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Bioinformatics studies of eukaryotic pathogens are an important part of developing new drug targets and diagnostics for diseases such as malaria, chagas, sleeping sickness and compromising diarrhea. The Eukaryotic Pathogen Database Resource (<http://eupathdb.org>) integrates genome sequence and annotations with functional genomics data in searchable databases for: *Babesia and Theileria* ([piroplasmadb.org](http://piroplasmadb.org)); *Crithidia, Endotrypanum, Leishmania, and Trypanosoma* ([tritypdb.org](http://tritypdb.org)); *Cryptosporidium* ([cryptodb.org](http://cryptodb.org)); *Eimeria, Gregarina, Neospora, and Toxoplasma* ([toxodb.org](http://toxodb.org)); *Encephalitozoon, Enterocytozoon, Nematocida, Nosema, Octospora, and Vivria* ([microspoidiadb.org](http://microspoidiadb.org)); *Entamoeba* ([amoebadb.org](http://amoebadb.org)); *Giardia* ([giardiadb.org](http://giardiadb.org)); *Plasmodium* ([plasmodb.org](http://plasmodb.org)); and *Trichomonas* ([trichdb.org](http://trichdb.org)). The specific advantage of the EuPathDB family of databases lies in the breadth of pathogens represented (78 organisms), the intuitive graphic web-interface, the extensive repertoire of pre-built searches, and the search strategy system that brings the power of genomics to novice users. Databases are updated and expanded bimonthly with comprehensive data sets ranging from genome sequence and annotations to gene and protein expression data to field isolates of parasites. Multistep search strategies are built one step at a time choosing from more than 90 pre-built searches, and successive searches can easily be combined in ways that refine the biological meaning of results. Our extensive user-support system includes on-line video tutorials, an email hotline for questions that receive a response within 48 hours, and hands-on workshops at locations worldwide. EuPathDB's user-friendly search strategy system embedded in full and up-to-date databases offers researchers a powerful tool for revealing meaningful biological relationships during computational experiments that support hypothesis-driven research. Attend this talk/poster for an overview of this resource. Hands-on demonstrations and help are available at our booth in the exhibit hall.

### EVALUATION OF THE ANTIMALARIAL ACTIVITY OF KOLAVIRON IN *PLASMODIUM BERGHEI* - INFECTED MICE

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Malaria is a major health problem in the world in general and in Sub-Saharan African countries. However, it is also becoming more difficult to treat malaria due to increasing drug resistance. Therefore, the need for discovery of alternative drugs is urgent and paramount. In order to study the antimalarial activity of Kolaviron (KV), Kolaviron was extracted from powdered seeds of *Garcinia kola* using methanol and chloroform. *Plasmodium berghei* was inoculated into Swiss albino mice. The mice (n=46) were infected with  $1 \times 10^7$  parasites intraperitoneally. The extracts were administered by an intra gastric tube daily for four days commencing after establishment of the parasite in the mice. The control group (n=10) received the same amount of corn oil (vehicle used to dissolve extract) while two groups received respectively 100mg/kg KV and 200mg/kg KV extract (n=10). The remaining two groups received chloroquine (10mg/kg body weight) and arteether (3.2mg/kg/body weight) and served as the standard reference drugs. All drugs were administered through the oral route. All animals were monitored for parasitemia and PCV changes for 7 days. Blood samples and liver homogenate were prepared and used for enzyme assays. Data were compared statistically between the groups and p<0.05 was regarded as significant. Result showed that in *P.*

*berghii* infected mice treated with kolaviron (200mg/kg), the percentage parasitemia decreased significantly ( $p < 0.05$ ) compared to untreated control animals. However, there was significant ( $p < 0.05$ ) increase in PCV levels of the parasite-infected CQ-treated, AE-treated, 100mg KV-treated and 200mg KV-treated groups post-treatment. However, kolaviron (200mg/kg) has a protective effect against liver and brain damage induced by malaria parasite. Kolaviron exerted antimalarial activity comparable with chloroquine and arteether by decreasing parasitemia and causing increase in PCV that suggests its haemopoietic effect. It is not toxic.

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### IDENTIFICATION OF ANTI-MALARIA BIOACTIVE COMPOUNDS FROM NAMIBIAN MEDICINAL PLANTS

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Medicinal plants are used to treat malaria and its symptoms such as febrile illness in rural settings in Namibia where access to health facilities is a challenge. It is important to study the efficacy of these indigenous treatments to validate their use and to investigate them as potential sources of new antimalarial compounds. Resistance and reduced sensitivity to ACTs have been reported, making such research imperative. Namibia is in the pre-elimination phase of malaria and an increased number of febrile illnesses are due to microbial infections. The objectives of this study are to identify anti-malaria compounds from selected Namibian plants and to determine the antimicrobial effect of extracts from medicinal plants. *V. infausta*, *G. coleosperma*, *M. sericea*, *Z. mucronata*, *A. inflata*, *O. dregeanum*, *P. angolensis* *D. mesipiliformis* and *Z. marlothii* were selected using secondary sources, survey data and literature review of medicinal plants in Namibia. Plant roots, bark and leaves were harvested and aqueous and organic extracts were prepared for analysis. Thin layer chromatography was used for phytochemical analysis, whilst column chromatography was used to fractionate the plant extracts for bioassays on *P. falciparum* 3D7 culture *in vitro*. The antimalarial compounds will be further analyzed with high performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR). Qualitative screens for antifungal, antibacterial, protease inhibitor and antioxidant assays were also carried out. Phytochemical analysis showed the presence of antimalarial compounds including, anthraquinones, flavonoids, terpenoids and steroids in the plants; *A. inflata* did not have terpenoids and steroids whilst *O. dregeanum* did not have flavonoids. All the plants showed significant antioxidant activities whilst all the plants exhibit anti-bacterial activities except for *V. infausta*. However, *O. dregeanum* did not show anti-fungal activities. All the plants exhibited protease inhibitory properties. Bioassay guided fractionation, HPLC, MALDI-MS and NMR will be used to identify the antimalarial compounds of interest.

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### CHEMOGENOMIC APPROACH TO IDENTIFY AND VALIDATE THE TARGET OF A DIVERSITY-ORIENTED SYNTHESIS PROBE

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The emergence and spread of drug resistance to antimalarial therapies remains a pressing concern with reports of artemisinin-based treatment failures escalating the need for novel antimalarial chemotherapies. The availability of complete genome sequences of different *Plasmodium* species and comparative bioinformatics have divulged several metabolic pathways for antimalarial drug discovery and genome-wide methods for target identification and understanding mechanisms of resistance. We have adopted a chemogenomics approach for identifying highly potent bioactives that can be powerful probes of parasite-specific biological

processes. Here we present studies of a novel probe from the Diversity-Oriented Synthesis Informer Set (DOS-IS) library with sub-nanomolar activity against the parasite in phenotypic whole cell assays. The DOS-IS is a representative collection of the >100k DOS compounds that have been synthesized at the Broad Institute. Compounds that are derived for DOS pathways aim to cover chemical space extending beyond the common confinements set by "drug-like" parameters, which is characteristic for traditional MedChem libraries and limits the diversity of represented compounds. We successfully applied an intermittent selection protocol to isolate Dd2 parasites that were 100-fold less sensitive to lead compounds in order to investigate the mode of action and molecular target of the DOS probe. To identify the genetic changes that confer resistance, we employed a whole-genome sequencing approach comparing the resistant mutants to the Dd2 parental line. These studies have led to the identification of target mutations in the Qi site of cytochrome b. The DOS mutants remain fully sensitive to atovaquone, suggesting that cross-resistance to both Qi and Qo site inhibitors might be challenging for the parasite and represent a promising avenue for the development of combination therapies. Further studies to determine the effects of targeting multiple active sites in a single enzyme and the ability of the parasite to develop dual resistance are underway.

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### NOVEL SERIES OF TRIAZINES FOR MALARIA TREATMENT AND CHEMOPROPHYLAXIS

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Malaria is the number one infectious disease threat for deployed US troops. There is a critical need for the discovery of new classes of anti-malaria drugs due to the therapeutic challenges emanating from the emergence of resistance in many structural classes of current therapies. A thorough retrospective analysis of Walter Reed Army Institute of Research's compound collection has identified a substituted triazine series that may be a single dose partner drug with minimal potential for resistance. Robust structure activity relationships (SAR) were established using *in vitro* blood stage assays with >250 analogs, wherein a number of compounds have been shown to possess oral *in vivo* efficacy in a modified Thompson assay. An early lead, exhibits excellent *in vitro* potency, excellent selectivity relative to two mammalian cell lines and complete cures (5/5 mice) as a single 160 mg/kg PO dose highlighting the potential for single dose cures. The series features a short chemical synthesis that will allow for rapid SAR development and a low cost of goods. The profile of this early lead and our approach to develop this series will be presented.

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### STUDIES TO ELUCIDATE THE MECHANISMS OF ACTION OF BENZOXABOROLES AGAINST *PLASMODIUM FALCIPARUM*

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There is an urgent need for new antimalarials with novel mechanisms of action. We have identified several series of boron-containing compounds with excellent *in vitro* potency and *in vivo* efficacy (for AN3661 IC<sub>50</sub> 37 nM against W2-strain *Plasmodium falciparum*; ED90 0.58 mg/kg and 0.3 mg/kg against murine *P. falciparum* and *P. berghei* respectively). We are investigating the mechanisms of action of benzoxaboroles against *P. falciparum*, focusing on inhibition of protein synthesis and of leucyl tRNA synthetase (LeuRS), as leuRS is an antimicrobial target of other benzoxaboroles. AN3661 and 9 other benzoxaboroles of various scaffolds

were tested in assays of stage-specificity, inhibition of protein synthesis, and inhibition of plasmodial leuRS activity. The trophozoite stage was most sensitive to AN3661. 8h incubation of synchronous trophozoites with 370nM AN3661 inhibited parasite development by 80%, compared to 40% for rings and 10% for schizonts. Inhibition of protein synthesis was assessed by comparing the incorporation of  $^{14}\text{C}$  leucine by parasites treated with test compounds or controls. Inhibition of cytoplasmic and apicoplast LeuRS from *P. falciparum* extracts was studied by including *S. cerevisiae* and *E. coli* tRNA, respectively in biochemical assays. In preliminary studies a dose-dependent inhibition of protein synthesis and LeuRS activity was observed for two related benzoxaboroles, but not AN3661. We also selected for *P. falciparum* with decreased sensitivity to AN3661 by culturing W2 parasites in increasing concentrations of the compound. Parasites were selected with ~100-fold decreased sensitivity ( $\text{IC}_{50}$  4  $\mu\text{M}$ ). Interestingly, some of the other benzoxaboroles shared the marked loss of activity against the selected parasites, while others demonstrated only a modest change or no difference in activity between initial and selected parasites, suggesting different mechanisms of action for different compounds. In summary, benzoxaboroles are a promising new class of antimalarial compounds, and preliminary studies suggest more than one antimalarial mechanism of action.

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### NO CLINICALLY RELEVANT ADVERSE EVENTS CORRELATED WITH PLASMA ARTESUNATE OR PLASMA DIHYDROARTEMISININ EXPOSURE PARAMETERS FOLLOWING SINGLE- OR MULTIPLE-DOSE OF ADMINISTRATION OF ARTESUNATE, OR ADMINISTRATION OF ARTESUNATE TO PATIENTS WITH UNCOMPLICATED PLASMODIUM FALCIPARUM MALARIA

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Artesunate (AS) is an artemisinin derivative which along with its active metabolite dihydroartemisinin (DHA), has activity against all erythrocytic stages of *Plasmodium falciparum* and currently in use as first line treatment in SE Asia against severe malaria. The metabolism of AS involves esterase cleavage in the plasma to DHA. This conversion also occurs via various cytochrome P450 enzymes (most notably CYP2A6). The pharmacokinetic and pharmacodynamic parameters of this drug and its active metabolite were reviewed with data from three previous studies (two safety and tolerability dose ranging phase I studies and one safety and efficacy phase II study in adults with uncomplicated malaria). Pharmacokinetic parameters including concentration of AS at initial injection ( $\text{AS } C_0$ ), AS area under the curve ( $\text{AS AUC}_{0-\infty}$ ), DHA maximum concentration ( $\text{DHA } C_{\text{max}}$ ), and DHA area under the curve ( $\text{DHA AUC}_{0-\text{last}}$ ) were compared to adverse events considered possibly or probably related to the study drug including: neutropenia, leucopenia, hypotension, pyrexia, infusion site pain, bradycardia, vomiting, hemoglobinuria, and anemia. This was done using a logistical regression analysis plot of the parameters and comparing the values to the incidence of the adverse events. No increase in frequency of adverse events was statistically correlated to increased level of any pharmacokinetic parameter measured. Thus concluding that both AS and its active metabolite DHA are equally well tolerated and safe at efficacious doses of parent drug.

## 679

### ENANTIOSELECTIVE PHARMACOKINETICS OF PRIMAQUINE BY HEALTHY HUMAN VOLUNTEERS

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Primaquine (PQ), the drug of choice for radical cure of relapsing *Plasmodium vivax* malaria, is currently used as a racemic mixture approximating a 50:50 ratio of (+) and (-) enantiomers. Earlier reports and recent studies have indicated differential therapeutic profiles of PQ enantiomers. Enantioselective pharmacokinetic, pharmacodynamic & pharmacologic characteristics may contribute to differential therapeutic indices of PQ enantiomers. A study was conducted with healthy adult human volunteers (age 26-51 years, with different racial/ethnic backgrounds) to determine plasma PK profile of enantiomers of PQ and carboxyprimaquine (cPQ), the major plasma metabolite. The individuals were orally administered three tablets of primaquine phosphate (equivalent to a total dose of 45 mg primaquine base) (Sanofi-Aventis US) 30 min after a normal breakfast. Blood samples were collected at different time intervals after administration of PQ and plasma samples were analyzed using LC-MS for enantiomers of PQ & cPQ. Plasma PQ levels were low and variable for both parent enantiomers and peaked around 2-4 hrs. Peak (-)-PQ levels ranged from 31-131 ng/mL. Peak (+)-PQ levels ranged from 21-146 ng/mL, around 2-4 hours. cPQ levels were much higher and surprisingly consistent from subject to subject, considering the variability in the parent levels. The peak levels of cPQ were observed at 8 hr (1200 ng/ml). However, very high levels were still present at 24 hr. This is consistent with earlier published studies on PQ pharmacokinetics. The key finding in this study was that essentially all of the cPQ detected was (-)-cPQ. (+)-cPQ was two orders of magnitude lower than (-)-cPQ, and in most samples it was only detected under the limit of quantification. The results suggest a markedly more rapid metabolism of (-)-PQ to (-)-cPQ than (+)-PQ. Alternatively, the (+)-PQ or (+)-cPQ could be rapidly converted to another metabolite(s) or distributed to tissues. This study confirms enantioselective pharmacokinetic and metabolic profiles of PQ and supports further clinical evaluation of PQ enantiomers.

## 680

### IN VITRO AND IN VIVO ANTIMALARIAL EVALUATION OF TIGECYCLINE IN COMBINATION WITH CHLOROQUINE

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Tigecycline (Tygacil®) is the first clinically available drug in a new class of antibiotics called "Glycylcycline". It is a semi-synthetic derivative of Minocycline with a unique and novel mechanism of action in bacteria. Several antibiotics have shown promising antimalarial effects and may be useful for malarial chemotherapy in combination with standard antimalarial drugs. Recently, tigecycline was tested against culture-adapted strains as well as clinical *Plasmodium falciparum* isolates from Lambaréné, Gabon, as reported previously. Tigecycline was found to act faster against the malaria isolates than any of the other antibiotics tested. Tigecycline was also tested against clinical isolates of *P. falciparum* from Bangladesh as reported previously. These study demonstrate the potential of tetracycline derivatives in the development of improved antimalarials and prompted us to evaluate the antimalarial potential of tigecycline *in vitro* against CQ sensitive and resistant strains of *P. falciparum* and also *in vivo* in *Plasmodium berghei* - mouse malaria model. The antibiotic was also tested

in combination with chloroquine (CQ). Tigecycline was more active against CQ-resistance (CQ-R) W2 strain than CQ-susceptible (CQ-S) D6 strain of *P. falciparum*. The chloroquine & tigecycline combination was selectively synergistic against the CQ-R (W2) strain, while the CQ susceptibility of CQ-S (D6) strain of *P. falciparum* was unaffected in combination with tigecycline. Further, treatment of *P. berghei* infected mice with Tigecycline (i.p.) caused significant suppression in parasitemia development and prolonged the mean survival time. Tigecycline in combination with suboptimal doses of chloroquine produced complete cure in *P. berghei* infected mice. These results support further evaluation of tigecycline as a potential combination candidate for treatment of drug-resistant cases of malaria.

## 681

### HIGH THROUGHPUT/HIGH CONTENT *IN VIVO* SCREENING OF ANTIMALARIAL HITS AS A SOURCE OF INNOVATIVE DRUG DISCOVERY PROGRAMS

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The recent disclosure of thousands of compounds active *in vitro* against the erythrocyte stage of *Plasmodium falciparum* is a major breakthrough for malaria eradication. However, the high number of potential starting points of drug discovery programs challenges the methods available to identify compounds that can lead to antimalarial medicines. In this work we show the feasibility of a new strategy to select efficacious, orally bioavailable and non-toxic compounds as optimal starting points of drug discovery projects based on a *P. berghei* high-content/high-throughput *in vivo* screening assay. This assay was developed by allometric extrapolation of human parameters of efficacy into mice. The assay is robust, minimizes the use of mice per compound (two mice) and allows detecting compounds able to stop parasite replication or to induce parasite killing *in vivo*. Finally, circa 600 compounds selected from the Tres Cantos Antimalarial set (TCAMs) active *in vitro* against *P. falciparum* were tested at 50 mg/Kg per oral route in an assay format that allows the evaluation of hundreds of compounds per month. The rate of compounds with detectable efficacy was about 10 %, of which about 33 % were as efficacious as marketed antimalarials. Our results support that the systematic high-content/high-throughput *in vivo* screening of compounds active *in vitro* against *P. falciparum* is a feasible strategy to rapidly select compounds with efficacy comparable to marketed antimalarials as starting points of drug discovery programs. This new paradigm is expected to accelerate the development of new antimalarial drugs.

## 682

### *IN VITRO* EVALUATION OF COMBINATIONS TO MITIGATE HEMOLYTIC TOXICITY OF PRIMAQUINE

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Primaquine (PQ) is the only drug of choice for radical cure of relapsing *Plasmodium vivax* malaria and also a useful drug combination for malaria prophylaxis & prevention of transmission. However, PQ has limited utility due to its narrow therapeutic index, particularly hemolytic

toxicities in G6PD deficient individuals. The reactive redox active metabolites generated through cytochrome P450-dependent pathways are regarded as responsible for hemolytic effects of primaquine and other 8-aminoquinolines. The hemotoxic response of the redox active metabolites of PQ, generated *in situ*, could be measured by accumulation of methemoglobin, kinetic measurement of increase in oxidative stress and depletion of reduced glutathione (GSH) in a microsomal metabolism-linked hemotoxicity assay. Several drugs, which are known to replenish intracellular levels of reduced thiols and/or protect the cells from oxidants injuries, were tested for mitigation of hemotoxic effects of potential toxic metabolites of PQ. N-Acetyl cysteine (NAC) has been reported to produce increase in intracellular GSH, decrease in oxidative stress and increase in erythropoietin (EPO) production. NAC was observed to partially attenuate the hemotoxic effects of 5-hydroxy PQ, a potential hemotoxic metabolite. NAC prevented the 5-HPQ induced accumulation of methemoglobin and oxidative stress and also protected the G6PD deficient erythrocytes from depletion of GSH. Lipoic acid and ascorbic acid also prevented 5-HPQ induced methemoglobin accumulation but did not protect the G6PD deficient RBCs from depletion of GSH. Some CYP inhibitors were also tested for attenuation of hemotoxic response of PQ in a microsomal metabolism linked hemotoxicity assay. The CYP3A4 and CYP2D6 inhibitors produced only partial attenuation. Chloroquine, which has earlier been reported to potentiate efficacy of PQ did not show any effect on PQ induced hemotoxic response *in vitro*. This study may help in developing a rational approach for developing suitable combinations for improving therapeutic utility of primaquine and other 8-aminoquinolines.

## 683

### DEVELOPMENT OF A NOVEL CHEMICAL SERIES WITH ACTIVITY AGAINST BOTH BLOOD- AND LIVER-STAGES OF *PLASMODIUM FALCIPARUM*

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Recent progress toward the development of a novel compound series with promising *in vitro* efficacy against both blood- and liver-stage *Plasmodium falciparum* will be described. Following the failure of one such compound to cure malaria-infected mice, focus has been to enhance the pharmaceutical properties of the compound series. Newer analogs, incorporating structural changes that enhance these properties, have been prepared. The *in vitro* efficacies and pharmaceutical properties of these will be described, including the kinetics of *in vitro* microsomal degradation, and the subsequent identification of predicted metabolites.

## 684

### MALARIA ELIMINATION IN THE PHILIPPINES: HOW MUCH DOES IT COST?

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Although numerous countries around the world are considering moving toward malaria elimination, there is little guidance as to how to make this transition and what resources would be needed. This study aims to (1) estimate the costs associated with sub-national malaria control programs as they progressively eliminate malaria over time and (2) understand how different environmental and organizational factors influence those costs. The Philippines offers a unique opportunity for conducting an expenditure study on malaria elimination because of its variety of epidemiological and ecological settings, and devolved health system. The country has also adopted a progressive, sub-national malaria elimination strategy unlike national-level strategies of other eliminating countries in the region.



Through key informant interviews and archival record retrieval in five select provinces - Apayao, Benguet, Cavite, Laguna, and Sorsogon we collect all expenditures associated with provincial malaria elimination programs for at least two years in each province (select years in 1998/99 and 2006/07, based on data availability) to enable comparisons across provinces, across years, and across program phases. Costs are gathered from a program perspective and include contributions (cash, in-kind) from the national government, donors, NGOs, and other private sector partners, but exclude private household expenditures. Preliminary results indicate that overall expenditures per population at risk decrease as programs progress from elimination to prevention of reintroduction, are generally higher when financed with international donor funding, and may be related to the level of economic development within a province. Ongoing data collection seeks to validate and complete these expenditure data as well as gather contextual information to understand these relationships in more depth. Lessons learned from the Philippines' malaria elimination efforts would fill a much-needed gap in information about what the strategies, interventions and financing requirements are to successfully eliminate malaria.

## 685

### A CLUSTER-RANDOMIZED TRIAL TO DETERMINE THE IMPACT OF HOTSPOT-TARGETED INTERVENTIONS ON MALARIA TRANSMISSION

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Malaria transmission is highly heterogeneous with some households being disproportionately exposed to malaria-infected mosquitoes. These households experience the highest malaria burden and are also hypothesized to contribute disproportionately to onward malaria transmission. Interventions targeted to these hotspots of malaria transmission may be an effective way to reduce malaria transmission at community level. We aimed to determine this community-wide effect of hotspot targeted interventions in a cluster-randomized trial in Rachuonyo, western Kenya. Hotspots were defined as areas for which there was strong evidence ( $p < 0.05$ ) of an elevated prevalence and density of combined AMA-1 and MSP-1 antimalarial antibodies. The presence of these hotspots was confirmed by parasite prevalence PCR. In the period preceding the transmission season of 2012, hotspots were targeted with mass distribution of long lasting insecticide treated nets, indoor residual spraying, larviciding and a focal screen and treat campaign where a sentinel age-group was screened for parasites and the entire household of parasite-positive individuals was treated with a curative dose of antimalarials. The intervention is being evaluated during cross-sectional surveys conducted in the transmission season (June-September). The methodology of the intervention will be presented, together with an analysis plan to estimate the extent of the community impact of hotspot-targeted interventions. Results from a first evaluation surveys will be presented.

## 686

### EXAMINING THE IMPACT OF COMBINING MULTIPLE INTERVENTIONS FOR MALARIA CONTROL BY USING A SPATIAL AGENT-BASED MODEL

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Malaria is a priority public health problem today. To reduce the disease burden, an essential objective is the scale-up application of proven, evidence-based, mosquito control interventions. The selection of interventions should consider local epidemiological parameters, some

of which may vary greatly. For example, insecticide-treated nets (ITN/LLIN) need the development of community-based distribution strategies seeking high coverage. Indoor residual spraying (IRS) is more appropriate in areas of unstable or epidemic transmission (e.g. in the eastern African highlands), and usually requires detailed reconnaissance of the target area. Source reduction may be applicable in especially arid areas, with a limited number of clearly defined breeding sites (e.g. in parts of the Horn of Africa). In some cases, even a single method of intervention may impact the transmission on multiple fronts. A recent study shows that multiple vector management scenarios, in combination, are 100 fold more effective in reducing transmission than any single measure used in isolation. Recently, much interest is observed on using multiple interventions in unison, with the expectation of achieving a synergistic effect caused by them. However, more detailed studies are required to analyze the efficacy of potential combinations of interventions before actually carrying out the more costly and time-consuming empirical evaluations. We have developed a spatial agent-based model (ABM) of malaria that simulates the vector dynamics lifecycle, including various stages of the dominant vector species *Anopheles Gambiae*. The ABM provides opportunities for more realistic modeling of important spatial events, such as bloodmeal seeking and oviposition. We also developed a landscape simulator to simulate landscapes used by the mosquito vectors. Using the spatial ABM and the landscape simulator simultaneously, we can investigate the impacts of multiple interventions for malaria control, and select the optimum mix of interventions for specific locations of interest. For example, initial results of our model show that carefully selected combinations of source reduction and ITNs yield better impacts than either of these interventions applied alone. The overall goal of our modeling effort is to quantitatively measure the effectiveness of multiple interventions and to demonstrate the model's ability to discover the synergistic benefits of using them.

## 687

### MALARIA CONTROL AND ELIMINATION IN SRI LANKA: DOCUMENTING PROGRESS AND SUCCESS FACTORS IN A CONFLICT SETTING

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As malaria transmission declines and malaria programs shift their focus from malaria control to elimination, it is vital to have documentation of the strategies that countries have used and are currently applying as they seek to eliminate malaria. Our case study of Sri Lanka, which has a long history of malaria control, including a period of near elimination and resurgence in the 1960s, aims to capture the key factors behind the country's decline in malaria over the last decade. The case study employed qualitative and quantitative methods, using data triangulation to compare and contrast trends. A literature review was conducted, and district and national data were collected on incidence, surveillance and vector control. Trends were observed across years and districts, in particular comparing conflict and non-conflict districts. Thirty-three key informant interviews were conducted. Expenditures in two districts for two years were compiled to identify changes in expenditure. Malaria incidence in Sri Lanka has declined by 99.9% since 1999. During this time, there were major increases in the proportion of malaria infections due to *Plasmodium vivax*, and those occurring in adult males. New vector control strategies were introduced, such as spatial insecticide rotation and long-lasting insecticide-treated nets. A strong passive case detection system is the foundation for diagnosis, while active case detection grew from identifying 1.1% of all infections in 2000 to 13.1% in 2007. Vector control and surveillance measures were maintained in conflict areas. For example, coverage of indoor residual spraying of risk populations in conflict districts was 45.9%

in 2005 (10.9% in non-conflict districts). One of two districts in the study reported a 48% decline in malaria programme expenditure per person at risk from 2004 to 2009, and a decline in prevention costs and an increase in surveillance costs. Malaria is now at low levels in Sri Lanka - 124 indigenous cases were found in 2011. Evidence-driven policy and an ability to adapt to new challenges contributed to this decline.

## 688

### EVALUATION OF THE EFFICACY OF IFAKARA ODOUR-BAITED STATIONS AGAINST MALARIA TRANSMISSION IN SOUTHERN TANZANIA: RESULTS FROM BASELINE MOSQUITO SURVEILLANCE

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Large-scale implementation of indoor residual spraying (IRS) and long lasting insecticide nets (LLINs) have significantly decreased malaria transmission across Africa. However, elimination remains elusive since interventions target mainly indoor transmission; thus the need for complementary outdoor interventions. Here we present baseline mosquito-surveillance data from a study designed to assess community-level effects of the Ifakara odour-baited station, a new outdoor intervention that lures and kills malaria mosquitoes. From a population of 2433 households in 3 villages (Kivukoni, Minepa and Mavimba) in southern Tanzania, 1600 households were randomly selected and spatially assigned, based on latitudes, to 16 clusters each consisting of 100 households. Monthly mosquito collections were performed using CDC-Light traps inside 6 households randomly selected from each cluster. The mosquitoes were sorted by taxa and abdominal status, after which a sub-sample of the malaria vectors were examined by (PCR) to distinguish between sibling species. The vectors were also examined by (ELISA) to detect *Plasmodium* sporozoites in their salivary glands. A total of 9,549 *An. gambiae* s.l and 2,529 *An. funestus* s.l were collected in the 3 villages during the first 5 months of the survey (Nov-2011 to March-2012). The distribution of *An. gambiae* s.l and *An. funestus* was spatially clustered, mostly in a set of adjoining clusters centered around the middle of the study area. At least 75% of *An. gambiae* s.l and 86% of *An. funestus*, were collected in adjoining clusters 7 to 14, centered in Minepa village. PCR and ELISA analyses are yet to be completed. These preliminary results show that most of the malaria vectors were collected from a set of contiguous clusters in an area centered in Minepa village, suggesting suitability of spatially targeted intervention. Further assessments are underway to determine risk factors associated with this distribution pattern and mosquito house entry, prior to introduction of the Ifakara odor-baited station.

## 689

### SERA FROM *PLASMODIUM VIVAX* PATIENTS BOOSTS *P. FALCIPARUM* GAMETOCYTE PRODUCTION *IN VITRO*

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We have previously described higher rates of gametocyte carriage in Cambodian patients who are found to relapse with *Plasmodium vivax* following treatment for *P. falciparum*. This led to the hypothesis that *vivax* coinfection boosts gametocyte production in patients with *falciparum* malaria. We sought to test this hypothesis *in vitro* by comparing gametocyte production in *P. falciparum* erythrocytic cultures grown in media containing sera from *P. vivax* patients vs. pooled naïve sera. NF54 *falciparum* parasites from the same culture flask were inoculated into microtiter wells and maintained with daily media changes containing 30% sera from either Peruvian individuals acutely infected with *P. vivax* or

control sera from malaria-naïve donors. Daily blood smears were examined for parasitemia and the development of early stage gametocytes. Over the 4 days of culture, parasitemia among the cultures grown in sera from *vivax* patients was slightly decreased compared to the control. On the other hand, gametocytogenesis occurred at a faster rate in the cultures exposed to *vivax* sera, with a significant difference in proportion of gametocytes by Day 5. At Day 5, in those cultures exposed to *vivax* sera, 37% of parasites were early stage gametocytes (range 22% to 78%) vs. 11% in the control cultures (range 9% to 14%) ( $p=0.03$ ). A similar trend was seen in cultures exposed to 20% sera from *vivax*-infected patients. Similar results with sera from Cambodian *vivax* patients will be presented, with support from expression data using real-time reverse transcriptase PCR targeting the early-stage gametocyte antigen, Pfg377. If these *in vitro* findings reflect the *in vivo* environment within hosts with mixed *P. falciparum/P. vivax* infection, the implications are that *vivax* coinfection may facilitate transmission of *falciparum* malaria in settings where the species are coendemic and improved control of *P. vivax* may also aid *P. falciparum* control efforts.

## 690

### THE FEASIBILITY OF MALARIA ELIMINATION IN SOUTH AFRICA

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Although malaria is still regarded as one of the most important public health burden on the African continent, in South Africa the disease is on the brink of elimination. The last major South African malaria epidemic occurred in 2000 where over 64 000 cases and approximately 500 deaths were reported. During this epidemic most of the cases and death were reported in KwaZulu-Natal, while Limpopo Province accounted for the lowest number of malaria cases and deaths. A decade on, the national number of malaria cases declined to less than 5000 with 44 reported malaria related deaths. Over this ten year period a change in malaria epidemiology was noted. Limpopo Province, adjacent to Zimbabwe, now accounts for most of the malaria cases and KwaZulu-Natal the least. This study involved a retrospective analysis of malaria case and mortality data collected by the malaria information system from 2000 until 2011, with the aim of determining of whether South Africa in the elimination phase of malaria. Our analysis revealed that two of the three malaria endemic provinces, namely KwaZulu-Natal and Mpumalanga Province have achieved the elimination phase cut-off threshold of <1 case per 1000 population. However, Limpopo Province is still in the consolidation phase of malaria control with an incidence of 1.68 cases per 1000 population. These results suggest that South Africa as a country is not eligible for elimination. However, if the malarious areas are partitioned according to their provincial status then both KwaZulu-Natal and Mpumalanga are well into the elimination phase and could eliminate malaria by 2018 as anticipated by the National Department of Health. Unfortunately due to the sustained high levels of malaria transmission in Limpopo Province it is still in the control phase. Data from this study suggests elimination certification for South Africa should be done at a lower level, ideally provincial, rather than at a national level. Moving forward the provincial malaria control programmes need to conclusively determine whether malaria cases are local or imported as well as identify and reduce local foci of transmission. It is also essential that cross-border initiatives are strengthening thereby decreasing imported malaria and local transmission.

## 691

### COMMUNITY PERSPECTIVES ON OUTDOOR MALARIA TRANSMISSION AND ITS CONTROL IN RURAL TANZANIA

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Despite the extensive use of indoor residual spraying (IRS) and insecticide treated nets (ITNs), residual malaria transmission persists in many parts of

Africa, partly driven by mosquitoes that bite people outdoors. Outdoor mosquito control devices such as mosquito traps have been proposed as complementary outdoor interventions. Even though outdoor mosquito control devices are clearly worth pursuing, it is essential to also consider the perspectives of a typical user- so as to ensure its sustainability. We assessed the views and behaviors of rural and peri-urban communities in southern Tanzania, regarding outdoor mosquito bites and malaria prevention. A qualitative cross sectional study was conducted in two rural villages (Lupiro and Minepa and one peri-urban village (Lipangalala) located within the Kilombero Valley in southern Tanzania, using semi-structured interviews and structured observation. A total of 30 participants were randomly selected for interview and their houses were observed. A prototype outdoor mosquito control device, was used to assess responses towards such outdoor interventions. Focal areas included a) whether malaria mosquitoes also bite outdoors and whether transmission can also occur and b) means of protection from mosquito bites while they outdoors. Preliminary results suggest that whereas people in these study area appreciate outdoor mosquito bites, most of them still believe that transmission mainly occurs indoors and at midnight. We observed numerous outdoor activities e.g. playing, fetching water storytelling, cooking, shopping and socializing at times when local malaria vectors are known to be most active outdoors. These are risk factors associated with outdoor malaria transmission. Overall, the communities were willing to use and contribute towards financing of the outdoor devices to control malaria. Further analysis is underway and results will be presented at the meeting. Providing well developed outdoor mosquito control devices that are acceptable will contribute substantially in reducing malaria transmission

## 692

### VALIDATION AND OPTIMIZATION OF NEW COMMUNITY BASED METHOD FOR IDENTIFYING SUITABLE AREAS TO LOCATE OUTDOOR MOSQUITO CONTROL DEVICES IN SOUTHERN TANZANIA

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Outdoor devices for luring and killing malaria vectors have been proposed as potential complementary interventions alongside existing insecticide treated nets (ITNs) and house spraying with residual insecticides (IRS). To enhance effectiveness of such outdoor interventions, it is essential to optimally locate them in such a way that they target most of the outdoor mosquitoes. We conducted a study to identifying suitable areas to locate outdoor mosquito control devices (Ifakara odor baited stations) one of the outdoor device developed at Ifakara Health Institute. The study was conducted in three villages (Kivukoni, Minepa and Mavimba) in Southern Tanzania. Maps of the villages were produced and sub divided into square grids (200m x 200m). In each village an area covering 300 grids was delineated for study. Using the village gridded maps and GPS to locate and mark all 300 sampling points per village. A list of random unique codes of all the grids (1 to 300) was produced. The trapping devices will be run for 12 hours during the night. In each village sample 300 grids every month and this processes replicate continuously over twelve (12) months so as to cover both dry and rainy seasons. Community meeting in the three villages was conducted where focus group discussion on mosquito life cycle, distributions and malaria transmission was conducted. Participants were provided with a gridded village map and pen, then were asked to rank all the grids by indicating on the grid, the likelihood (on a scale of 1-5) based on their own experiences and knowledge. Five being high number, followed by 4, 3, 2 and one being lowest number of mosquitoes. The data obtained will be imported to ArcGIS 9.3 standard Desktop and display using the ArcMap application. Overall, the communities know where the mosquitoes are abundant. Further analysis is underway and results will be presented at the meeting. Optimal location of developed outdoor mosquito control devices will significantly reduce mosquito biting and outdoor malaria transmission.

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### IDENTIFYING AND CHARACTERIZING FOCI OF MALARIA TRANSMISSION IN POTENTIAL MALARIA ELIMINATION SETTINGS IN RURAL SENEGAL

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Sparse distribution of malaria morbidity has become a consistent feature of the disease, particularly in the new context of malaria decline. Foci of residual transmission (hot spots) are particularly relevant to malaria control programme in countries such as Senegal with low transmission intensity, since their presence makes it very difficult to eliminate the disease. Based on the 2008 and 2009 general changes on the epidemiological profile of the disease and in particular on the very low incidence rates observed all over the sub-region (figure), we designed this project to identify and characterize the foci of residual transmission and to pilot methods for surveillance that could be used as part of an elimination programme. Scientific research questions were therefore the following: 1. Has transmission stopped in some areas? a) Evaluation of the validity of health facilities records b) Use of serology to determine if transmission of malaria has stopped in defined area. 2. Why is transmission persisting in some places? Several factors could explain the patchy distribution of malaria (use of preventive measures, housing, environmental or climatic factors). Several activities were conducted to answer to the research questions listed above among which: a) A collection and analyses and validity assessment of 2008-2011 health facilities records. b) A case-control study with a community survey around all cases and controls intervention coverage's in the vicinity of where the cases and the controls live. c) Cross sectional surveys to assess level of malaria transmission from serology d) An environmental evaluation in and in areas with low transmission Data analysis is on going and results will be presented at the conference.

## 694

### THE USE OF LOT QUALITY ASSURANCE SAMPLING (LQAS) TO ASSESS MALARIA PARASITE PREVALENCE AND GUIDE MASS SCREENING AND TREATMENT CAMPAIGNS

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As malaria parasite prevalence declines, the utility of standard probability household surveys to measure malaria parasite prevalence decreases for ascertaining relatively precise point estimates. Other survey methods are needed to evaluate malaria interventions and provide estimates of the malaria burden once the prevalence has decreased below 10%. One alternative is lot quality assurance sampling (LQAS), which has been used to define areas as low or high depending upon a threshold set by the surveyor. LQAS has been adapted to the health sector in low and middle income countries as a tool to identify areas with adequate health services of various types, including an application in estimating areas of low and high malaria parasite prevalence. It has the potential to be a useful tool in identifying villages as low and high malaria parasite prevalence, as well as identifying areas of low and high vector control coverage. We used census data from a mass malaria screening and treatment campaign in Southern Zambia to assess the feasibility of using LQAS to identify villages with low malaria parasite prevalence (malaria parasite prevalence <10% ) and high ITN coverage (>50% of households within a catchment area owning at least 1 ITN). We ran simulations using the census data to ascertain the sensitivity and specificity of LQAS sampling for randomly

selected individuals and households to detect villages with low malaria parasite prevalence and high ITN coverage respectively. Preliminary results suggest that LQAS is both sensitive and specific at detecting areas with low malaria parasite prevalence and high ITN coverage, and that gains in sensitivity and specificity can be made by sampling individuals 6-18 years of age when classifying villages as low malaria prevalence. It is envisioned that at the outset of dry season malaria screening campaigns, in areas with heterogeneous levels of malaria transmission, LQAS can be employed at local levels to assist classification of catchment areas into mass or more focal screen and treat activities.

## 695

### EXAMINATION OF SURVEILLANCE DATA MILESTONES AS ZANZIBAR TRANSITIONS TO THE PRE-ELIMINATION OF MALARIA, 2008-2011

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Zanzibar introduced artemisinin-based combination therapy and intermittent preventive treatment of malaria for pregnant women in 2003 and 2004, respectively, followed by insecticide treated bednets for pregnant women and children under 5 years of age in 2005, and indoor residual spraying of households in 2006. This comprehensive package of interventions has led to *Plasmodium falciparum* prevalence estimates <1% in population-based household surveys since 2008. Specific surveillance indicators with milestones are provided by the World Health Organization to guide programmatic transition from malaria control to pre-elimination to elimination. Transition from malaria control to pre-elimination occurs once the slide or rapid diagnostic test (RDT) positivity rate is consistently <5% among febrile patients. Criteria for transition to the elimination phase includes case reporting from all health facilities and malaria incidence reduced to <1 case per 1000 persons at risk per year. The Zanzibar Malaria Control Programme has collected and analyzed weekly malaria surveillance data from all 142 government health facilities since 2008. Approximately 90% of these facilities use a *P. falciparum* histidine-rich protein-2 based RDT to confirm presence of malaria in febrile outpatients. Between 2008 and 2011 the number of diagnostic tests performed on outpatients at all 142 health facilities increased 130% (from 115,361 to 265,403 tests). The annual test positivity rate among febrile outpatients declined from 3.5% in 2008 to 1.2% in 2011. The weekly test positivity rate exceeded 5% on four separate occasions (10 weeks total) in 2008, but from January 2009 through April 2012 the 5% threshold was exceeded on only four occasions (seven weeks total). Annual malaria incidence among the entire population (1.3 million in 2011) declined from 3.3/1000/yr (95% confidence interval [CI], 3.2-3.4/1000/yr) to 2.4/1000/yr (95% CI, 2.3-2.5/1000/yr) between 2008 and 2011, respectively (27% reduction). Zanzibar is nearing the pre-elimination phase of malaria control, particularly during 2009-11 when the weekly test positivity rate rarely exceeded 5%. Annual malaria incidence in Zanzibar remains 2-3 fold higher than required for transition to the elimination phase.

## 696

### AFRICAN WOMEN'S PERIPHERAL BLOOD MONONUCLEAR CELL COMPOSITION IS ALTERED BY *PLASMODIUM FALCIPARUM* INFECTION INDEPENDENTLY OF GESTATIONAL AGE OR DELIVERY

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Placental malaria is caused by sequestration of *Plasmodium falciparum* infected erythrocytes in the intervillous spaces of the placenta, resulting in pathological alterations. There are, however, limited data concerning the profiles of immune cells associated with malaria physiopathology during pregnancy. As part of the STOPPAM study described here, we performed *ex vivo* assays using flow cytometry to evaluate the impact of pregnancy-associated malaria (PAM) on the phenotypic composition and activation status of peripheral blood mononuclear cells (PBMC) and to identify PBMC profiles that are associated with malaria outcomes. STOPPAM was a longitudinal, prospective study conducted in Benin and Tanzania, in which 1000 pregnant women with a gestational age below 24 weeks, were enrolled at each site and followed up until delivery. Infected women were identified through examination of thick and thin blood smears. The phenotypes and activation status of PBMC were analysed in samples of subgroups of 131 women (62 infected and 69 uninfected) at inclusion and 111 women (37 infected, 27 'exposed' and 47 uninfected) at delivery in the Benin cohort. Verification of the findings generated by these analyses was sought through identical analyses of PBMC in subgroups of women from the Tanzanian cohort. At inclusion, the frequencies of B cells and their expression levels of the activation marker CD86 were higher ( $p=0.04$ ;  $p=0.01$ ), whilst other parameters such as the expression of HLA-DR on antigen-presenting cells (monocytes, dendritic cells) and the frequency of regulatory T cells were lower ( $p=0.01$ ) in PBMC of infected versus uninfected women. In *P. falciparum*-infected women, anaemia was associated with a decreased frequency of peripheral blood monocytes. At delivery, PBMC of uninfected women had fewer low expressing CD86<sup>+</sup> B cells, more plasmacytoid (p)DC, more myeloid (m)DC expressing high levels of HLA-DR, and fewer T effector cells (CD4<sup>+</sup>CD25<sup>+</sup>CD127<sup>+</sup>) compared to those with infection with respective *p-values* ( $p=0.03$ ,  $p=0.01$ ,  $p=0.008$ ,  $p=0.02$  and  $p=0.007$ ). The differences in the PBMC profiles at inclusion compared with delivery will be discussed in the context of possible differences in the duration of the *P. falciparum* infections detected at the two time-points.

### CONSEQUENCES OF MALARIA DURING PREGNANCY ON NEONATAL ANTIGEN PRESENTING CELL ACTIVATION VIA TOLL-LIKE RECEPTOR LIGAND AND ON *PLASMODIUM FALCIPARUM* ANTIGENS RESPONSES IN BENIN

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During pregnancy and early childhood, there is an increased susceptibility to malaria due to parasite-induced modulation of pro-inflammatory responses. The development of a protective immune response requires correct function of Toll-like receptors (TLR) expressed by antigen-presenting cells (APC). TLR activation induces cytokine production and expression of co-stimulatory molecules to trigger antigen presentation to T cells. In newborns, stimulation of APC via their TLR is involved in the progressive development of immune responses during the first months of life and our hypothesis is that pregnancy-associated *Plasmodium falciparum*-malaria may adversely affect this development. The study is part of the STOPPAM project conducted in Benin; a clinical and parasitological follow-up of 200 pregnant women and their children followed from birth to 12 months. To assess the impact of malaria on neonatal immunity peripheral whole blood of children (0, 3, 6 and 12 months) was stimulated either by TLR ligands (polyI:C, LPS, R848, CpGODN) that have distinct effects on APC subsets. Concentrations of pro- and anti-inflammatory cytokines were then evaluated in culture supernatants to investigate activation levels of APC. We performed the stimulations and cytokine assays on blood from 134 newborns from mothers with different malaria histories during pregnancy in order to evaluate the impact of an *in utero* contact with *P. falciparum* antigen on the newborn immune system. As a general trend, the responses of APC to TLR stimulation increased with age. According to a multivariate analysis, the mother's infection at delivery had a significant effect on infant immune responses: higher concentrations of IL6 in response to CpGODN and polyI:C at birth; of IL-10 in response to CpGODN and polyI:C at 3 and 12 month; of TNF- $\alpha$  in response to CpGODN, polyI:C and R848 at 6 months. Our findings suggest that mother's malaria infection at delivery altered the neonatal innate immune responses via TLR activation that may have consequences in the development of immune responses.

### ANTIGEN-SPECIFIC CYTOKINE RESPONSES TO *PLASMODIUM FALCIPARUM* DURING A PERIOD OF SUSTAINED LOW MALARIA TRANSMISSION IN A HIGHLAND AREA OF KENYA

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Acquisition and maintenance of protective immunity to malaria relies on the frequency of exposure. Despite the global scale-up of malaria control that has realized a reduction in transmission, the longevity of antigen-specific cellular immune responses to *Plasmodium falciparum* in areas of very low transmission is not well characterized. In addition, most studies

assessed responses to one or two cytokines and not to multiple cytokines that may be involved in protection from *P. falciparum* infection or disease. We assessed levels of pro-and anti-inflammatory cytokines in response to the *P. falciparum* pre-erythrocytic (CSP, LSA-1, TRAP) and blood-stage (AMA-1, MB2, MSP-1) antigens in 100 individuals from a highland area of western Kenya with unstable malaria transmission. Responses were assessed from April 2008- April 2009, following a 14-month period from March 2007- April 2008 during which no malaria transmission was observed. Malaria incidence during this period was very low, with an incidence of 20% of responses to most *P. falciparum* antigens were seen for IFN- $\gamma$ , RANTES and TNF- $\alpha$ , whereas lower frequencies of responses (<20%) to most *P. falciparum* antigens were seen for the IL5, IL-6 and IL-10. Three distinct patterns of immune responses to *P. falciparum* antigens emerged, with some variation by antigen: a significant decrease in immune response during the first 6 months that was sustained at 12 month follow-up (IFN- $\gamma$ , TNF- $\alpha$  and IL-10), a significant decrease during the first 6 months, followed by a modest increase at 12 month follow-up (IL-5), and no change over time (IL-6, RANTES). Patterns did not differ between pre-erythrocytic and blood-stage antigens. In the absence of high-level malaria transmission, IFN- $\gamma$ , TNF- $\alpha$ , IL-5 and IL-10 responses to *P. falciparum* decrease, while IL-6 and RANTES responses are stable. Future studies will assess whether loss of specific immune responses is associated with loss of clinical immunity to malaria.

### PF332-C231-REACTIVE ANTIBODIES AFFECT GROWTH AND DEVELOPMENT OF INTRA-ERYTHROCYTIC *PLASMODIUM FALCIPARUM* PARASITES

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The *Plasmodium falciparum* antigen 332 (Pf332), is a megadalton parasite protein expressed at the surface of infected red cells during later stages of the parasite's developmental cycle. Antibodies to different parts of this antigen have been shown to inhibit parasite growth and adherence to host cells with or without ancillary cells. However, the mechanisms involved in these inhibitions remain largely unknown. We further analysed the activities of specific antibodies with regard to their specific mechanisms of action. For these analyses, affinity purified human antibodies against epitopes in the C-terminal fragment of Pf332 (Pf332-C231) were employed. All purified antibodies recognized Pf332-C231 both by immunofluorescence and ELISA. IgG was the main antibody isotype detected, although all sera investigated had varying proportions of IgG and IgM content. All the antibodies showed a capacity to inhibit parasite growth in *P. falciparum* cultures to different extents, mainly by acting on the more mature parasite stages. Morphological analysis revealed the antibody effects to be characterized by the presence of a high proportion of abnormal schizonts (15-30%) and pyknotic parasites. There was also an apparent antibody effect on the red cell integrity, as many developing parasites (up to 10% of trophozoites and schizonts) were extracellular. In some cases, the infected red cells appeared to be disintegrating/fading, staining paler than surrounding infected and uninfected cells. Antigen reversal of inhibition confirmed that these inhibitions were antigen specific. Furthermore, the growth of parasites after 22-42 h exposure to antibodies was investigated. Following the removal of antibody pressure, a decreased growth rate of these parasites was seen compared to that of control parasites. The present study confirms the potential of Pf332 as a target antigen for parasite neutralizing antibodies, and further indicates that epitopes within the C231 region of Pf332 should constitute important tools in the dissection of the role of Pf332 in the biology of the malaria parasite, as well as in the design of a malaria vaccine.

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### LONGITUDINAL DYNAMICS OF FUNCTIONAL AND SEROLOGIC ANTI-MALARIA ANTIBODY RESPONSES IN KENYAN INFANTS

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Maternal antibodies transferred to the fetus during pregnancy protect the infant from malaria infection. These antibodies are thought to wane by 6-9 months of age after which infants slowly generate their own anti-malaria antibodies in response to repeated infections. In this study we measured anti-malaria antibodies in plasma samples from a longitudinal cohort of infants from 2006-2008 (Kisumu District, Kenya) with blood samples drawn approximately every month from birth to 30 months of age. Plasma samples from 22 infants (~17 blood samples/infant) were examined for the presence and magnitude of anti-malaria antibodies by a) serology to 11 malaria antigens, b) human antibody recognition of variant surface antigens exported to the erythrocyte membrane from 4 different parasite strains, and c) functional antibody-mediated growth inhibition of cultured parasites. Differential patterns of anti-malaria antibody waning and waxing were observed. Serologic measured antibodies directed against the majority of antigens tested (AMA1, MSP1, EBA140, EBA175, EBA181, LSA1, PfCSP, PfCelTOS) waned by 6-12 months of age. For all antigens tested except EBA175, infant antibody levels and prevalence reached or exceeded birth levels by 18-32 months of age. The most robust responses were observed with AMA1 and LSA1. Growth inhibitory antibodies were measured from birth to 12 months of age and observed at low levels throughout this time course which peaked between 6 and 9 months of age. Antibodies directed against variant surface antigens reached a nadir at 12 months and slowly climbed from 12-30 months of age. Infant variant surface antibody levels and prevalence did not reach birth levels. Infants with evidence of recent malaria infection (defined by positive blood smear, PCR or IgM responses to more than 5 antigens) had some evidence of boosting of antibodies directed against variant surface antigens, but not serologically measured anti-malaria antibodies. Whether these antibodies are markers of exposure or protection is not clear.

## 701

### RAPID DEPLETION OF B AND T LYMPHOCYTES DURING *PLASMODIUM YOELII* 17XNL MURINE MALARIA IS ACCOMPANIED BY A SELECTIVE GAIN IN ACTIVATED CD69<sup>+</sup> CD4<sup>+</sup> AND CD8<sup>+</sup> T CELLS AND ANTIBODY SYNTHESIZING CD19<sup>+</sup>CD138<sup>+</sup> PLASMABLASTS

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To better understand the mechanisms operational during the formation of malaria immunity, we constructed a detailed immunological profile throughout the course of a *Plasmodium yoelii* 17XNL infection. At peak parasitemia (day 9), infection was marked by a rapid depletion of TCR $\alpha\beta$ <sup>+</sup> T cells. There was a 50% decrease ( $p < 0.0001$ ) in the frequency of both CD4<sup>+</sup> and CD8<sup>+</sup> T cells compared to uninfected mice. However, despite this apparent loss, there was a significant increase ( $P < 0.0001$ ) in activated (CD69<sup>+</sup>) CD4<sup>+</sup> and CD8<sup>+</sup> T cells indicating that this loss could be due to activation induced cell death (AICD) - a well defined mechanism of cell death in which activation through the T cell receptor results in apoptosis. There was also a 35% decrease in CD19<sup>+</sup> B cells at peak parasitemia compared to uninfected mice. Despite this substantial loss in B cells, there was a significant increase in the frequency of antibody synthesizing

CD19<sup>+</sup>CD138<sup>+</sup> plasmablasts that was maximal (9.2%) at peak parasitemia. A proinflammatory response, marked by the presence of IFN- $\gamma$ <sup>+</sup>CD4<sup>+</sup> T cells and the IFN- $\gamma$ , IL12p70 and KC cytokines, was observed during the ascending phase (day 6) of infection. In contrast, an anti-inflammatory response, characterized by production of the IL-5, IL-6 and IL-13 cytokines, was induced during the clearance phase of infection (day 13). Finally, maximal levels of TCR $\gamma\delta$ <sup>+</sup> T cells (6.8%) and Ly6g<sup>+</sup> neutrophils (2.8%) were observed at day 13, indicating a possible role for these cells in parasite clearance. Ongoing studies are directed to define the antigen specificity for the loss of splenic T and B cells and to understand the mechanism of the switch from a Th1 to Th2 response

## 702

### INDIVIDUAL AND EPISTATIC EFFECTS OF GENETIC POLYMORPHISMS OF CD40, CD40L AND BLYS GENES, CO-STIMULATORY MOLECULES ON SUSCEPTIBILITY TO *PLASMODIUM VIVAX* MALARIA

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Following the candidate gene approach we analyzed the *CD40*, *CD40L* and *BLYS* genes that participate of B-cell co-stimulation, for association with *Plasmodium vivax* malaria, characterized by a non-lethal disease but its prolonged and recurrent infection with deleterious effects on personal well-being, growth and on the economic performance at individual, family, community, and national levels. *P. vivax* is the most prevalent malaria species in Brazil it represents more than 80% of clinical cases reported annually from the Amazon region. The parasite-host coevolutionary process can be viewed as an arms race, in which adaptive genetic changes in one are eventually matched by alterations in the other, in this case, within the genetically diverse Amazonian populations. The sample included 97 patients and 103 controls. We extracted the DNA by using the extraction and purification commercial kit and identified the SNPs -1 C>T in the gene *CD40*, -726T>C in the gene *CD40L* and the -871C>T in the gene *BLYS* by the PCR-RFLP method. We analysed the genotypic, allelic frequencies, as well as of those individuals carrying each allele, by direct counting. We also compared the observed genotypic frequencies with the expected ones, according to the Hardy-Weinberg Equilibrium. The allelic, genotypic and allele carrier frequencies for these SNPs did not differ statistically between the patient and the control groups. Gene-gene interactions were not observed between *CD40* and *BLYS*, and between *CD40L* and *BLYS*. Overall, the genes were balanced according to Hardy-Weinberg Equilibrium. The results of this study lead us to conclude that, although the *CD40*, *CD40L* and *BLYS* alleles analysed differ functionally, this variation does not alter the functionality of the molecules in a way that would interfere with the susceptibility of the disease.

## 703

### REDUCED ANTIBODY RESPONSES AGAINST *PLASMODIUM FALCIPARUM* VACCINE CANDIDATE ANTIGENS IN THE PRESENCE OF *TRICHURIS TRICHIURA*

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Helminths may affect immune responses to vaccines. In the target group for malaria vaccines helminth prevalence is high. Twenty Gabonese preschool-age children were vaccinated with GM22, a blood stage malaria

vaccine candidate. Humoral immune response against the vaccine antigens and parasitological status were assessed. Antibody response to GMZ2 was 3.4-fold (95% confidence interval: 1.6, 7.4) higher in *Trichuris trichiura* negative subjects compared to positive participants. Immunoglobulin subclass distribution was similar, whereas memory B-cell response tended to higher in *T. trichiura* negative individuals. Future malaria vaccine development programs need to account for worm-mediated hyporesponsiveness of immune reactions.

## 704

### IMPACT OF IPTC IN ANTIBODIES MSP-119 AND AMA-1 IN SENEGAL

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Malaria remains a major disease in many African countries, caused an estimated 243 million cases of clinical malaria and 863 thousand deaths globally 2008. The acquisition of immunity to clinical malaria is usually acquired the first five years of life depending on the intensity of malaria transmission. Nowadays, many strategies such as IPTic /SP are used for prevention in children. Intermittent Preventive Treatment for children (IPTc) against *Plasmodium falciparum* malaria is administered at defined intervals curative doses independently of the presence of parasites or symptoms. IPTc could however delay the acquisition of the antibodies which are managed against the malaria to this group of children. In this optics we want to understand the impact of this strategy in kinetics of specific antibodies against malaria on the acquisition of antibody in children living in zone of unstable transmission. This study measure the kinetics of antibodies MSP-119 and AMA-1 by ELISA, which are recombinant proteins specifically managed against the membrane of *P. falciparum*. To evaluate the impact of IPTic /SP on antigenic variation in rural areas of three districts, all children aged 11mths-10years, in Senegal. Our results show that young children under 5 years are the ones who produce most antibodies and this production increases significantly with age ( $p=0, 0001$ ). Production of AMA-1 antibody is more important (27, 09 %) than MSP-119 antibody (13, 69 %). Control zone produce more antibodies than intervention zone, the PSP is a factor which can modifies the production of antibody. Seroepidemiology can provide key information on malaria transmission for control programmes, when parasite rates are low.

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### ELEVATED LEVELS OF CD4+CD45RA-CD62L+CD11A+ ARE ASSOCIATED WITH MALARIA SEVERITY IN CHILDREN FROM WESTERN KENYA

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High endothelial venules from secondary lymphoid organs display high levels of CD62L (L-selectin) ligands. In addition, murine T cells that undergo activation in secondary lymphoid organs up-regulate CD11a (LFA-1), an adhesion molecule that mediates interaction with activated endothelia and recruitment of activated T cells to inflamed regions. However, the role of CD11a and CD62L in the development of malaria severity remains largely unexplored. As such, we characterized CD4+ T-cell populations in parasitemic children (n=54; aged 12-36 months) presenting with varying severities of malarial anemia at Siaya District Hospital (SDH), in western Kenya. Complete hematological profiles were obtained with a Beckman Coulter Counter®, while Giemsa-stained slides were used to determine parasitemia. Participants were stratified based

on hemoglobin (Hb) status as uncomplicated malaria (UM; Hb $\geq$ 11.0 g/dL; n=12), mild malarial anemia (M/MA; Hb $\geq$ 8.0<11.0 g/dL; n=22), and severe malarial anemia (SMA; Hb<6.0 g/dL; n=20). Venous blood was stained with anti-(CD3; CD4; CD45RA; CD11a and CD62L) antibodies. Cells were then acquired using four-color FACSCalibur™ flow cytometer. These results demonstrated that the proportion of (CD4+CD45RA-CD11a+) were comparable across the groups [median (IQR) UM, 98.16% (1.97); M/MA, 97.41% (2.71); SMA, 97.43% (2.69);  $P=0.422$ ]. In addition, the proportions of (CD4+CD45RA-CD62L+) was comparable across the groups [median (IQR) UM, 72.52% (11.90); M/MA, 76.88% (12.80); SMA, 77.33% (11.90);  $P=0.229$ ]. However, the co-expression of CD11a and CD62L (CD4+CD45RA-CD11a+CD62L+) differed significantly across the groups [median (IQR) UM, 67.98% (5.96); M/MA, 75.46% (15.07); SMA, 72.17% (9.89);  $P=0.025$ ]. Further analysis showed that both the M/MA ( $P=0.012$ ) and SMA ( $P=0.040$ ) groups had elevated levels of circulating CD4+CD45RA-CD11a+CD62L+ relative to the UM group. These results suggest that CD4+ T cells co-expressing CD11a and CD62L are associated with malaria severity in this holoendemic transmission area, and may be important in the pathogenesis of SMA.

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### VARIATION IN THE HSPA1A GENE LOCUS IS ASSOCIATED WITH SUSCEPTIBILITY TO SEVERE MALARIAL ANEMIA

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Heat shock protein (HSP) 70 is an important stress-inducible protein known to play a dual role as a molecular chaperone and immune modulator. Under normal physiological conditions, HSPs are constitutively expressed at low levels, but show dramatically increased expression during cellular stress and infection. Although polymorphic variation in the genes encoding HSP70 protein have been implicated in the pathogenesis of several diseases, their role in *Plasmodium falciparum* malaria has not been reported. In this context, we investigated the functional role of polymorphic variations in the promoter region of the *HSP70* gene [i.e., *HSPA1A* -217C/G (rs1043618), -5457A/C (rs2227955), and -5893G/A (rs34814308)] in conditioning malaria disease pathogenesis in children from a holoendemic *P. falciparum* transmission region of western Kenya. Parasitemic children (n=855; aged 3-36mos.) were stratified according to hemoglobin (Hb) levels into severe malaria anemia [(SMA), Hb<5.0 g/dL] and non-SMA (Hb $\geq$ 5.0g/dL). Multivariate logistic regression analyses (controlling for covariates) demonstrated that the homozygous mutant (GG) at the -217 locus was associated with increased susceptibility to SMA [Odds ratio (OR), 2.736; 95% CI, 0.948-7.898;  $P=0.063$ ], while the heterozygous (GA) at the -5893 locus was protective against SMA (OR, 0.538; 95% CI, 0.307-0.943;  $P=0.030$ ). Haplotypic analyses revealed that carriage of the CAA (-217C/-5457A/-5893A) haplotype was associated with reduced risk of developing SMA (OR, 0.531; 95% CI, 0.298-0.947;  $P=0.032$ ) and elevated levels of *HSPA1A* transcripts ( $P=0.009$ ). Further examination of the relationship between malaria-associated inflammatory mediators and hsp70 regulated genes demonstrated that carriers of CAA haplotype had significantly lower production of IL-1 $\beta$ , IL-6 and TNF- $\alpha$  ( $P<0.05$  for all). Taken together, these findings demonstrate that variation in the *HSPA1A* promoter is associated with susceptibility to SMA and production of inflammatory mediators known to affect the pathogenesis of SMA.

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### REDUCED SYSTEMIC BICYCLO-PROSTAGLANDIN-E<sub>2</sub> AND CYCLOOXYGENASE-2 GENE EXPRESSION ARE ASSOCIATED WITH INEFFICIENT ERYTHROPOIESIS AND ENHANCED UPTAKE OF MONOCYTIC HEMOZOIN IN CHILDREN WITH SEVERE MALARIAL ANEMIA

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In holoendemic *Plasmodium falciparum* transmission areas, severe malaria primarily occurs in children <48 mos. and manifests as severe malarial anemia [SMA; hemoglobin (Hb) <5.0 g/dL]. Induction of high levels of prostaglandin-E<sub>2</sub> (PGE<sub>2</sub>) through inducible cyclooxygenase-2 (COX-2) is an important host defense mechanism against invading pathogens. We have previously shown that COX-2-derived PGE<sub>2</sub> levels are reduced in children residing in hyperendemic transmission regions with cerebral malaria and in those with mixed sequelae of anemia and hyperparasitemia. Our *in vitro* studies further demonstrated that reduced PGE<sub>2</sub> was due to down-regulation of COX-2 gene products following phagocytosis of malarial pigment (hemozoin, PfHz). However, since COX-2-PGE<sub>2</sub> pathways and the impact of naturally acquired PfHz on erythropoietic responses have not been determined in children with SMA, plasma and urinary bicyclo-PGE<sub>2</sub>/creatinine and leukocytic COX-2 transcripts were determined in parasitized children (<36 mos.) stratified into SMA (n=36) and non-SMA (Hb≥5.0 g/dL; n=38) groups. Children with SMA had significantly reduced plasma (P=0.001) and urinary (P<0.001) bicyclo-PGE<sub>2</sub>/creatinine, and COX-2 transcripts (P=0.007). There was a significant positive correlation between Hb and both plasma (r=0.363, P=0.002) and urinary (r=0.500, P=0.001) bicyclo-PGE<sub>2</sub>/creatinine. Furthermore, decreased systemic bicyclo-PGE<sub>2</sub>/creatinine was associated with inefficient erythropoiesis (i.e., reticulocyte production index; RPI<2.0, P=0.026). Additional analyses demonstrated that plasma (P=0.031) and urinary (P=0.070) bicyclo-PGE<sub>2</sub>/creatinine and COX-2 transcripts (P=0.026) progressively declined with increasing concentrations of naturally acquired PfHz by monocytes. Results presented here support a model in which reduced COX-2-derived PGE<sub>2</sub>, driven in part by naturally acquired PfHz by monocytes, promotes decreased erythropoietic responses in children with SMA.

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### THE EFFECT OF CHANGING MALARIA TRANSMISSION ON THE ACQUISITION AND MAINTENANCE OF IMMUNITY TO MALARIA IN PREGNANT MALAWIAN WOMEN

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Malaria in pregnancy can have severe effects on both the mother and the foetus, including severe anaemia, low birth weight, congenital malaria, abortion, as well as maternal or infant mortality. These severe manifestations are often accompanied by the accumulation of *Plasmodium falciparum* (Pf)-infected erythrocytes (IE), pigmented monocytes and fibrin deposits in the placenta. Studies have identified VAR2CSA PfEMP1-expressing Pf that bind to chondroitin sulfate A in the placenta. In areas of high transmission, susceptibility to placental malaria decreases with increased gravidity, suggesting a protective role for antibodies (Abs) generated against placenta-binding Pf. The prevalence of malaria in pregnant women has decreased in some regions over the last decade

with the increased use of insecticide-treated bed nets and intermittent preventative treatment during pregnancy. In this study, we investigate the impact of decreased malaria transmission on the acquisition of immunity to placental malaria (PM) and the maintenance of immunity to non-pregnancy malaria (NPM) among 500 pregnant women in Malawi over the period 1999 to 2006, when malaria prevalence decreased by approximately eighty per cent. To measure the acquisition of immunity to PM, we perform a number of assays to assess sera for protective Abs that bind to VAR2CSA PfEMP1-expressing Pf-IE, that prevent IE-placental adhesion and Abs that can opsonise IE for clearance by phagocytic cells. Maintenance of immunity against NPM during this period will be assessed by performing the same assays against CD36-binding PfEMP1-expressing Pf-IE, and by assaying for Abs against merozoite antigens.

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### ANTI-PARASITE ANTIBODY RESPONSE ELICITED BY *PLASMODIUM FALCIPARUM* UNCOMPLICATED MALARIA

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Malaria results in the death of hundreds of children and pregnant women every week in tropical countries where the disease is endemic. Nevertheless, repeated *Plasmodium falciparum* malaria episodes do confer immunity against parasite infection, reducing the severity and morbidity attributed to the disease. However, such anti-malarial immunity gradually subsides and is not well understood. In a prospective cohort study carried out at Kasangati Health Centre in Wakiso district of Uganda, patients with uncomplicated *P. falciparum* malaria were enrolled, consented, treated with policy regimen (artemether-lumefantrine), demographics and clinical data obtained, all on day 0. Venous blood was collected on the same day of diagnosis and treatment (day 0) and at 42 days later (day 42), filter-paper dot blots were prepared and serum immunoglobulin antibodies against synthetic peptides representing *P. falciparum* candidate antigens were evaluated by ELISA. The studied peptides correspond to antigenic domains within i) glutamine rich protein (GLURP), ii) merozoite surface protein 3 (MSP3), and iii) histidine rich protein II (HRPII). Two hundred and fifty patients of 1 to 60 years of age were enrolled. Anti-*P. falciparum* peptide IgG levels assessed on basis of ELISA absorbance increased with age, especially anti-GLURP peptide IgG. Indeed, mean anti-GLURP IgG significantly increased from day 0 to day 42 but anti-MSP3 and anti-HRPII IgG did show similar increase. Although the sample size of HIV infected enrolled patients was small, we noticed that the mean parasite density of patients with CD4 lymphocyte counts less than 200 CD4/uL was higher than the corresponding values for HIV-negative patients and HIV-infected patients possessing higher than 200 CD4/uL.



## CONSEQUENCES OF MALARIA DURING PREGNANCY ON IMMUNOLOGICAL RESPONSES OF THE NEWBORN: A STUDY OF REGULATORY T CELLS

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Characterization of the factors linked to the higher susceptibility of the newborns to *Plasmodium falciparum* infection is a priority for the validation of any anti-malarial vaccine. Regulatory T cells (Treg) part of the natural pool of CD25<sup>+</sup> CD4<sup>+</sup> T cells play a major role in the maintenance of peripheral tolerance to self-antigens, and in the regulation of adaptive and innate immune responses against pathogens by controlling inflammatory responses and lymphocyte homeostasis. Treg are characterized by the phenotype CD4<sup>+</sup> CD25<sup>+</sup> CD127<sup>-</sup> Foxp3<sup>+</sup>. Treg prevalence was observed to be higher in cord blood from a *P. falciparum* infected placenta, compared to uninfected placenta, with an effect on cellular immune responses, suggesting *in utero* stimulation on Treg by soluble *P. falciparum* antigens with altered pro-inflammatory responses. Our objective is to evaluate the impact of pregnancy associated malaria (PAM) and malaria during the first year of life on the profile of Treg at birth, and at 3, 6 and 12 month of age. Our hypothesis is that *in utero* contact with *P. falciparum* soluble antigens may modify the profile of Treg and we questioned the consequences of those changes on outcomes of infection during the first year of life. This study is part of the STOPPAM project a clinical and parasitological follow up of 1000 women during pregnancy and 200 infants during the first year of life in Comé, southwestern Benin. Immunophenotyping of Treg cells has been evaluated using flow cytometry in 158 infants in cord blood and again in peripheral blood at 3, 6 and 12 months of age from mothers with different malaria histories during pregnancy. Our results from a multivariate analysis show first, a significant increased of Treg frequency with infant age as described in newborn. Importantly, we observed a significant increased of Treg frequency at 3 month of age when the child was born of infected mothers at delivery. Our results suggest an impact of PAM on newborn cellular immune responses with an alteration in the control of pro-inflammatory responses necessary for the removal of parasites.

## CONSEQUENCES OF MALARIA DURING PREGNANCY ON NEONATAL ANTIGEN PRESENTING CELL ACTIVATION VIA TOLL-LIKE RECEPTORS AND *PLASMODIUM FALCIPARUM* ANTIGENS IN BENIN

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During pregnancy and early childhood, there is an increased susceptibility to malaria due to parasite-induced modulation of pro-inflammatory responses. The development of a protective immune response requires correct function of Toll-like receptors (TLR) expressed by antigen-presenting cells (APC). TLR activation induces cytokine production and expression of co-stimulatory molecules to trigger antigen presentation to T cells. In newborns, stimulation of APC via their TLR is involved in the progressive development of immune responses during the first months of life and our hypothesis is that pregnancy-associated *P. falciparum*-malaria may adversely affect this development. The study is part of the STOPPAM project conducted in Benin; a clinical and parasitological follow-up of 200 pregnant women and their children followed from birth to 12 months. To assess the impact of malaria on neonatal immunity peripheral whole blood of children (0, 3, 6 and 12 months) was stimulated either by TLR ligands (polyI:C, LPS, R848, CpGODN) that have distinct effects on APC subsets. Concentrations of pro- and anti-inflammatory cytokines were then evaluated in culture supernatants to investigate activation levels of APC. We performed the stimulations and cytokine assays on blood from 134 newborns from mothers with different malaria histories during pregnancy in order to evaluate the impact of an *in utero* contact with *P. falciparum* antigen on the newborn immune system. As a general trend, the responses of APC to TLR stimulation increased with age. According to a multivariate analysis, the mother's infection at delivery had a significant effect on infant immune responses: higher concentrations of IL6 in response to CpGODN and polyI.C at birth; of IL-10 in response to CpGODN and polyI.C at 3 and 12 month; of TNF- $\alpha$  in response to CpGODN, polyI.C and R848 at 6 months. Our findings suggest that mother's malaria infection at delivery altered the neonatal innate immune responses via TLR activation that may have consequences in the development of immune responses.

### PLASMODIUM FALCIPARUM CLEARANCE RATES IN RESPONSE TO ARTESUNATE IN MALIAN CHILDREN WITH MALARIA

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Artemisinin resistance, defined as a long half-life ( $T_{1/2}$ ) of parasite clearance in response to artesunate, was recently described in patients with *Plasmodium falciparum* malaria in Cambodia and Thailand.  $T_{1/2}$  values have not yet been reported from Africa, where artemisinin-based combination therapies were recently introduced. In Mali, we sought to establish a baseline parasite clearance rate in our study population and to investigate the contribution of acquired immunity to parasite clearance rates in response to artemisinins. In 2010 and 2011, we measured  $T_{1/2}$  in 215 Malian children aged 0.5-15 years with uncomplicated *P. falciparum* malaria (10,000 – 100,000 parasites/ $\mu$ l). We provided directly-observed, weight-based doses of artesunate (0, 24 and 48 h) and amodiaquine (72, 96 and 120 h) orally, and counted the parasite density ( $\mu$ l) in the blood every 6 hours until it was undetectable. From plots of  $\log_e$ -transformed parasite densities vs. time, we calculated  $T_{1/2}$  and evaluated the effects of age, sex, ethnicity and red blood cell (RBC) polymorphisms on this parameter. The geometric mean  $T_{1/2}$  was 1.93 hours (95% CI 1.85-2.01), significantly shorter than in western Cambodia (5.8 h). In a linear regression model that accounted for host factors,  $T_{1/2}$  decreased by 4 minutes for every 1-year increase in age ( $r=-0.3117$ ,  $p<0.0001$ ). For 48 children, we quantified the proportion of parasitized RBCs recognized by autologous plasma IgG. The proportion of parasitized RBCs recognized by autologous plasma IgG ranged from 4%-76%. IgG responses increased significantly with age ( $r=0.3831$ ,  $p=0.0072$ ) and correlated inversely with  $T_{1/2}$  ( $r=-0.475$ ,  $p=0.0006$ ). Our data indicate that acquired IgG responses to parasitized RBCs shorten  $T_{1/2}$  and suggest that clearance of *P. falciparum* is in part achieved by IgG responses. These IgG responses may also contribute to  $T_{1/2}$  values in areas like Southeast Asia, where age is an inadequate surrogate of immunity and no *in vitro* correlate of parasite-clearing immunity has been identified.

### KINETICS OF ANTIGEN-PRESENTING CELL POPULATIONS AFTER CONTROLLED HUMAN MALARIA INFECTION

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Antigen-presenting cells (APCs) are key players in the induction and regulation of immune responses via antigen-presentation, co-stimulation and production of cytokines and chemokines. In malaria, it remains elusive whether APCs, and particularly dendritic cells (DCs) are appropriately activated to subsequently carry out their function. In naturally exposed individuals living in endemic areas, blood DC activation seems to be impaired and *in vitro* studies suggest a weakened function of human

monocyte-derived DC after *Plasmodium falciparum* (*Pf*) exposure. On the contrary, other *in vivo* and *in vitro* experiments show activation of DCs by *Plasmodium*. In most studies, the effect of the malaria parasite on DCs seems highly dose-dependent. We investigated the effects of low numbers of *Pf* on APCs in a first encounter *in vivo*, over the course of a controlled human malaria infection. Fifteen healthy Dutch malaria-naive volunteers were infected by intradermal injection of cryopreserved *Plasmodium falciparum* sporozoites (PfSPZ Challenge). Peripheral blood mononuclear cells (PBMC) were collected and cryopreserved at baseline, at several time points during liver-stage and blood-stage of the malaria infection and on days 35 and 140 after infection. We analyzed the cells by phenotypic staining followed by 9-colour flow cytometry. We will present the kinetics in composition of the blood-APC compartment (including BDCA-1, BDCA-2, BDCA-3 and CD16 DC subsets as well as classical and non-classical monocytes) and changes in activation, co-stimulatory and chemokine marker expression in the context of human liver- and blood-stage malaria infection.

### HLA-DR4 MOLECULES SUPPRESS PROTECTIVE ANTIBODY RESPONSES TO MALARIA PY17XNL BLOOD STAGE PARASITES

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*Plasmodium falciparum* is the most virulent and deadly malaria parasite that infects annually >200 million people and accounts for more than 650,000 deaths. HLA-DR4 expression in humans infected with *P. falciparum* has been associated with severe malaria and high mortality rate. Herein, we show that humanized mice expressing HLA-DR4 molecules and lacking mouse MHC class II molecules (AbbKO mutation) have impaired production of specific antibodies to normally non-lethal *P. yoelii* 17XNL blood stage parasites and succumb to infection. In contrast, F1 hybrid mice co-expressing HLA-DR4 and mouse MHC-II molecules as well as mice expressing HLA-DQ6, HLA-DQ8 or HLA-DR3 molecules on AbbKO background, were able to elicit antibodies and to self-cure the infection. As compared to BALB/c, C57BL/6, and the F1 hybrids, the HLA-DR4.AbbKO mice had an increased frequency of CD4<sup>+</sup>Foxp3<sup>+</sup> regulatory T cells (Tregs) upon infection, and *in vivo* Treg depletion enabled them to elicit antibodies and self-cure the infection. These results demonstrated that HLA-DR4 expression associates with suppression of protective humoral responses to Py17XNL parasites. These results are consistent with clinical associations between HLA-DR4 and severe *falciparum* malaria.

### PROFESSIONAL ANTIGEN PRESENTING CELLS, NOT INFECTED HEPATOCYTES, INDUCE PROTECTIVE IMMUNE RESPONSES TO PLASMODIUM FALCIPARUM CSP IN P. BERGHEI TRANSGENIC MOUSE MODEL

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The role of Circumsporozoite Protein (CSP) in protection against malaria is controversial. While studies in rodent models indicated that CSP is not required for protection upon immunization with whole sporozoites -either irradiated or live under chloroquine cover- the role of *Plasmodium falciparum* CSP (PfCSP) upon vaccination of humans with whole parasites

is still uncertain. Also, the role of infected hepatocytes vs professional antigen-presenting cells in the presentation of PfCSP in an immunogenic form remains unclear. Here we show that mice injected intravenously with live *P. falciparum* sporozoites, which cannot infect mouse hepatocytes, elicited high titers of CSP-specific antibodies and were protected (>60%) against challenge with transgenic *P. berghei* sporozoites expressing PfCSP, but not against challenge with wild type *P. berghei* sporozoites. The results give evidence that PfCSP is a protective antigen, and highlight a critical role of professional antigen-presenting cells in stimulating protective immunity to PfCSP. This study supports efforts for using CSP subunit vaccines against human malaria.

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### STUDY OF ANTIBODY MEDIATED CORRELATES OF PROTECTION AGAINST MALARIA IN THE MOUSE MODEL

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A near full length circumsporozoite protein (CSP) of *Plasmodium falciparum* (Pf) was expressed in *E. coli*, and purified to homogeneity. Pf CSP contains an N-terminal region, a central repeat (R) region and a C-terminal cysteine rich region. While several studies have shown the R-specific antibodies are the primary mediators of protection, the N- and C-terminal region-specific antibodies have also been implicated in protection. In the present study we dissected the antibody response to CSP to determine if the quantity and quality of antibodies against CSP could predict protection in a transgenic *P. berghei* parasite challenge model. The transgenic parasite expresses the *P. f* CSP gene in a rodent malaria (*P. berghei*) parasite, as reported previously. Mice vaccinated with CSP using a variety of adjuvants and delivery systems were challenged with the transgenic parasites. Protection was inferred if blood stage parasites were absent 15 days post challenge. Antibody correlates that were examined included ELISA titers against the full length, repeat and C-terminal region of CSP, antibody isotypes analyzed by luminex, avidity by thiocyanate method, IFA on fixed sporozoites and inhibition of sporozoite invasion into hepatocytes. Monoclonal antibodies are also being produced against CSP for passive transfer and competition ELISA. The data indicated that antibodies contribute to protection and certain correlates could distinguish protected from non-protected mice. These data have broader implications for down-selecting improved CSP based vaccine formulations for human trials.

## 717

### TRANSFORMING GROWTH FACTOR B-1 LEVELS CORRELATE WITH PLATELET COUNT, RANTES LEVELS AND SEVERITY OF DISEASE IN *PLASMODIUM FALCIPARUM* MALARIA

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A balance between pro- and anti-inflammatory cytokines appears to be necessary to defend against and survive malaria. Transforming growth factor beta-1 (TGF- $\beta$ 1), a cytokine that regulates inflammation, has inflammatory properties at low levels and anti-inflammatory properties at higher concentrations. Platelets are a major source of TGF- $\beta$ 1, and platelet counts are reduced in severe malaria. However, the relationships between TGF- $\beta$  levels and disease severity, platelet count and levels of other cytokines and chemokines are not well described. To assess these relationships, serum levels of TGF- $\beta$ 1 were assessed by ELISA, and IL-1 $\beta$ , IL-6, IL-10, IFN- $\gamma$ , TNF- $\alpha$ , MIP-1 $\alpha$ , MIP-1 $\beta$ , and RANTES by cytometric

bead assay (CBA), in Ugandan children with cerebral malaria (CM, n=75), uncomplicated malaria (UM, n=67) or healthy community children (CC, n=62). TGF- $\beta$ 1 levels decreased with increasing severity of disease (median levels in pg/ml in CC, 44.0 (16.8-82.5), UM, 25.7 (0.8-70.6), CM, 14.1 (1.9-39.3), *P* for trend<0.0001). In children with CM or UM, TGF- $\beta$ 1 levels on admission correlated positively with platelet count (CM, Spearman's rho=0.61, *P*<0.0001, UM, rho= 0.38, *P*=0.0015), and RANTES levels (CM, rho= 0.56, *P*<0.0001; UM, rho= 0.48, *P*<0.0001). In children with CM, TGF- $\beta$ 1 levels on admission correlated negatively with IFN- $\gamma$  (rho=-0.39, *P*=0.001). TGF- $\beta$ 1 levels were not associated with death or with adverse neurologic or cognitive outcomes. The study data suggest that reduced levels of TGF- $\beta$ 1 and RANTES in severe malaria may be due to the low platelet count seen in severe malaria. In turn, reduced TGF- $\beta$ 1-mediated regulation of pro-inflammatory cytokines may lead to increased levels of IFN- $\gamma$  and other pro-inflammatory cytokines, and thus lead to more severe disease. Further research is required to elucidate the temporal relationships between these factors.

## 718

### APOPTOSIS OF NON-PARASITIZED RED BLOOD CELLS IN *PLASMODIUM YOELII* MALARIA

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Recently, through the study of erythrocytic apoptosis during *Plasmodium yoelii* infection, we observed a rise in the levels of non-parasitized red blood cells (nRBC) apoptosis that could be associated with the development of severe malaria anaemia, as premature elimination of nRBC is a relevant mechanism leading to this malaria complication. In the present study, we attempt to investigate the participation of nRBC apoptosis in malaria anaemia as well as the influence of parasite load and immune response on this cell death process. Balb/c mice were intraperitoneally infected with *P. yoelii* 17XL and, then, nRBC apoptosis as well as number of peripheral RBC, parasitemia and plasmatic levels of cytokines, nitric oxide and anti-RBC antibodies were evaluated at early and late stages of infection. Apoptosis of nRBC was increased only at the late stage of infection and it was related to parasite load, but not to the intensity of the immune response. In spite of increased percents of nRBC apoptosis observed when the anaemia degree was accentuated, this increase was not associated to the reduction of peripheral RBC counts. We conclude that nRBC apoptosis in malaria can be induced in response to high parasite load and that this apoptotic process does not significantly contribute to the anaemia observed in the malaria model studied herein. Further studies on malaria models in which acute anaemia develop under low parasite burden could help to identify the potential pathogenic role of nRBC apoptosis.

## 719

### DEVELOPING NOVEL SEROLOGIC ASSAYS OF MALARIA EXPOSURE AND PROTECTION

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Current methods for assessing *Plasmodium falciparum* (Pf) transmission intensity are labor intensive and inaccurate, limiting our ability to assess effects of control interventions. In particular, there are no standard biomarkers to assess exposure or immunity to malaria parasites. Antibody responses to Pf develop as a function of the number and timing of Pf exposures. Kinetics of antibody responses to specific Pf antigens have only been evaluated for a small subset of antigens, but have been shown to differ. Specific antibody responses are known to provide protection from malaria, but protective responses are poorly defined. We propose that assessment of antibody responses to appropriately selected Pf antigens will allow estimation of recent and cumulative exposure to Pf and estimation of protection from malaria. Previously, we probed a microarray containing ~23% of the Pf proteome with plasma from 220 subjects in Mali and identified serologic responses to 49 Pf proteins associated with protection. Here, we analyzed these microarray data to identify novel markers of prior exposure and additional biomarkers of protection to Pf. 47 antigens were identified as candidate markers for cumulative exposure by selecting responses with the best linear fit of increased antibody intensity with increasing age in children 2-10 years. 78 candidates for recent exposure were identified as responses with either the most significant difference in reactivity between the beginning and end of the malaria transmission season or the best ability to predict elapsed time since last parasitemia, assuming an exponential decay of antibodies. 49 additional candidates for protection were identified by variable importance indices in random forest analysis. Based on these analyses, an array with 185 unique proteins will be tested with longitudinal samples from Ugandan children in a wide variety of transmission settings in an attempt to translate estimates of Pf exposure and protection at an individual level to estimates of transmission intensity and immunity at a population level.

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### EVALUATION OF ANTIBODY TITERS TO *PLASMODIUM FALCIPARUM* AND *P. VIVAX* MEROZOITES SURFACE PROTEIN-1 (MSP-1) IN CAMBODIAN ADULTS DEVELOPING UNCOMPLICATED MALARIA DURING AN OBSERVATIONAL COHORT STUDY

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Merozoite surface protein-1 (MSP1), a protein involved in erythrocyte invasion, is found on the surface of the malaria blood stage merozoite. In addition to being a marker of past malaria exposure, there is evidence for a protective role of MSP1 antibodies against malaria infection. As part of an active, observational cohort with a nested treatment study evaluating the efficacy of dihydroartemisinin-piperaquine (DP) for uncomplicated malaria, we conducted a sero-epidemiological analysis

for the prevalence of antibodies to both *Plasmodium falciparum* and *P. vivax* MSP1 in service members of the Royal Cambodian Armed Forces between October 2010 and February 2011, typically considered a period of moderate to low seasonal transmission in Cambodia. Of 256 healthy adult volunteers screened, 222 were enrolled with 89 developing primary uncomplicated malaria infection with 32 volunteers having a recurrence after DP treatment. The majority of primary infections (75%) were *P. vivax* with the remaining 25% being *P. falciparum*. Using a titer of 450 units as a cutoff based on non-immune Thai adult blood donors, 76% and 56% of volunteers had titers above cutoff to *P. vivax* and *P. falciparum* respectively. Titers to Pv MSP1 at baseline ranged from 65-965,671 units with a geometric mean titer (GMT) of 2207 units (95% CI 1746-2789); the range of Pf MSP1 titers at baseline was 16-669,774 units with a GMT of 904 (95% CI 703-1162). For volunteers who were treated for malaria, Pf MSP1 titers at time of infection did not change, but titers for Pv MSP1 doubled, returning to baseline at discharge. Uninfected volunteers had a lower GMT at discharge than baseline, possibly reflecting less exposure at the end of the transmission season. The individual and grouped serological data will also be analyzed in relationship of antibody titer at baseline infecting genotype and species at time of infection as well as to recurrences after curative treatment with DP.

## 721

### ANTENATAL MALARIA INFECTIONS ARE ASSOCIATED WITH IMPAIRED HIB AND DIPHTHERIA VACCINE IMMUNE RESPONSES IN KENYAN CHILDREN

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Antenatal maternal parasitic infections prime the fetal immune response and induce an immunomodulatory phenotype at birth that may affect subsequent immune responses to commonly administered childhood vaccines. Here we examined the effect of malaria, schistosomiasis and filariasis in pregnant women on the responses to Haemophilus influenzae type B (Hib) and diphtheria (DT) vaccination in their offspring. 241 infants were followed every 6 months from birth to 3 years and IgG antibody levels to polyribitol-ribose phosphate (PRP Hib) and diphtheria toxoid (DT) were measured by ELISA. Linear mixed-effects models were used to characterize PRP and DT responses over 6 to 36 months of age. Offspring of malaria-infected women tended to have lower PRP levels compared to offspring of women without known malaria infection during pregnancy although none of these comparisons were significant. By contrast, classification of children based on their mother's malaria infection status and on cord blood (CB) recall responses to malaria blood-stage antigens as putative tolerant (mothers with malaria [Pf+] but lacking detectable Th1 and Th2-type recall responses to malaria-blood stage antigens, N=12), sensitized (Pf+ women and detectable Th1 and Th2-type recall responses in CB to malaria, N=153) or unexposed (mothers Pf- and lack of CB lymphocyte responses, N=75) had a significant impact on PRP- and DT-specific IgG levels. Putatively tolerant children had lower DT- and PRP-specific IgG levels at 24, 30 and 36 months of age to PRP (P=0.37, <0.04 and <0.005) and DT (P=<0.02, 0.006 and <0.04) compared to unexposed children. Initial analysis indicates maternal schistosomiasis and/or filarial infection during pregnancy may have less impact on PRP- and DT-specific IgG levels than that observed with maternal malaria infections. Thus, malaria during pregnancy may impair childhood vaccine efficacy and highlight the importance of programs to eradicate parasitic infections in pregnant women.

### FIRST EVIDENCE OF PYRETHROID RESISTANCE IN AN *ANOPHELES FUNESTUS* POPULATION FROM SENEGAL

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*Anopheles funestus* is one of the major malaria vectors in tropical Africa notably in Senegal. The highly anthropophilic and endophilic behaviours of this mosquito make it a good target for vector control operations through the use of insecticide treated nets, long-lasting insecticide nets and indoor residual spraying. However, little is known about the resistance status to insecticides of field populations of this vector in Senegal and the potential underlying resistance mechanisms. To fill this gap in our knowledge, we assessed the susceptibility status of *An. funestus* populations from Gankette Balla, located in the Senegal River Basin. WHO bioassays indicated that *An. funestus* in Gankette is resistant to lambda-cyhalothrin 0.05% (74.32% mortality / n = 222). Suspected resistance was observed to deltamethrin 0.05% (87.72% mortality / n = 114), permethrin 0.75% (91.37% mortality / n = 139), DDT 4% (93.20% mortality / n = 147), bendiocarb 0.1% (94.27% mortality / n = 157) and dieldrin 4% (96.41% mortality / n = 306). However this population is fully susceptible to malathion 5% (100% mortality / n = 50) and fenitrothion 1% (100% mortality / n = 55). Sequencing of the fragments of Voltage-Gated Sodium Channel did not detect the L1014F mutation. A microarray analysis indicated that the lambda-cyhalothrin resistance in Gankette is conferred by metabolic resistance mechanism under the control of P450 genes. This study represents the first report of pyrethroid resistance in *An. funestus* from Senegal. These findings should be taken into account by malaria control programs and further studies are needed to establish the geographic distribution of this resistance across Senegal.

### INCREASED LEVELS OF PYRETHROID RESISTANCE IN THE PRIMARY MALARIA VECTOR *ANOPHELES ARABIENSIS* IN RURAL LOWER MOSHI, NORTHEASTERN TANZANIA

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The major foci of pyrethroid resistance in 1990-2010 were in West and Central African populations of *Anopheles gambiae* s.s. In East Africa pyrethroid resistance has been recorded in relatively few locations and at low frequencies. Three cross-sectional surveys of *Anopheles arabiensis* were conducted to determine levels of resistance to organochlorines, organophosphates, carbamates and pyrethroids in Lower Moshi. Wild mosquitoes were hand-collected from cowsheds between April-June in 2004, 2009 and 2011 in several villages. Susceptibility tests were conducted using standard WHO diagnostic dosages and kits. Knockdown was recorded after 5, 10, 15, 20, 30, 40, 50, 60 minutes and mortality 24 h post-exposure was recorded. The first survey in 2004 of *An. arabiensis* indicated low levels of resistance to permethrin (84% mortality) and deltamethrin (87% mortality). *An. arabiensis* were fully susceptible to DDT (organochlorine), fenitrothion and malathion (organophosphates), and propoxur (carbamate). The 2009 survey data showed a slight increase in the frequency of resistance for lambda-cyhalothrin (mortality <80% in 3 villages), and permethrin (67% mortality in one the villages). The mean mortality for all villages tested after exposure to deltamethrin was 92%. The recent 2011 survey revealed high levels of resistance to lambda-cyhalothrin in all villages (mortality <60%) and deltamethrin in

Msitu wa tembo (31% mortality). Resistance to permethrin remained moderate in most villages and similar to 2009. *An. arabiensis* were still fully susceptible to DDT, carbamates and organophosphates. These results clearly demonstrate the presence of pyrethroid resistance in *An. arabiensis* in Lower Moshi. The lack of DDT resistance coupled with previous studies showing very low frequency *kdr* suggests that enzyme-based mechanisms are responsible for resistance in *An. arabiensis*. Tanzania has recently scaled-up vector control programmes with universal coverage of pyrethroid LLINs and IRS in several regions in 2011. Regional monitoring of resistance should continue and provide an early warning so that alternative insecticides can be considered if resistance levels become operationally significant

### EVIDENCE OF HIGHLY FOCAL PATTERNS OF PYRETHROID RESISTANCE IN *Aedes aegypti* IN IQUITOS, PERU

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Dengue fever is one of the most rapidly growing public health problems worldwide, with an estimated 50 million infections annually and 2.5 billion people living in areas at risk of infection. In the absence of a vaccine, control of dengue vector mosquitoes (principally *Aedes aegypti*) is the only available preventive measure. The city of Iquitos, located in the Amazon region of north-eastern Peru, is a highly dengue endemic city, with an estimated 60% of the population having been infected with at least one of the four dengue virus serotypes. Vector control activities rely heavily on the use of chemical insecticides to attack both larval and adult mosquito stages. The intensity of vector control responses to seasonal peaks in dengue transmission has increased in recent years, and the pyrethroid alphacypermethrin has been particularly heavily deployed as an adulticide during recent outbreaks. Although insecticide resistance was not previously reported in Iquitos, this intensive pyrethroid use appears to have increased selection pressure on local *Ae. aegypti* populations, as recent bioassay data indicate that pyrethroid resistance, particularly to lambda-cyhalothrin and deltamethrin, is increasing within the city, but is limited to certain foci. Bioassay data also show widespread resistance to DDT, suggesting that cross-resistance patterns between DDT and pyrethroids may also be focal. Resistance to carbamates and organophosphates has not yet been detected. Data will be presented in terms of geographical and temporal variation, resistance intensity, and intensity of insecticide-based control activities over 2 dengue transmission seasons.

### NOVEL INSECTICIDE BIOASSAY BASED ON SUGAR FEEDING BY ADULT *Aedes aegypti* (L.) (DIPTERA: CULICIDAE)

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Population monitoring to detect insecticide resistance in mosquitoes is an essential component of integrated disease management programs. We developed a bioassay method for assessing insecticide susceptibility based on the feeding activity of mosquitoes on plant sugars. The prototype sugar-insecticide feeding bioassay system was composed of inexpensive,

disposable components, contained minimal volumes of insecticide, and was compact and highly transportable. Individual mosquitoes were assayed in our feeding module consisting of a transparent plastic cup (30 mL) with a snap-on lid. The cup contained a feeding platform fitted with a receptacle holding a 10% sucrose-permethrin solution. Trypan blue dye was added to the sucrose-permethrin solution to create a visual marker in the mosquito's abdomen for ingested sucrose solution. Blue fecal spots deposited by a mosquito onto the feeding platform provided further evidence of solution ingestion. The proximity of the mosquito to the solution facilitated rapid ingestion. In time-course experiments, 100% of mosquitoes fed on the solution within 2 h of exposure. With the sugar-insecticide feeding bioassay, the permethrin susceptibility of *Aedes aegypti* females from two field-collected strains was characterized by probit analysis of dosage-response data. Further optimization of the device is possible to produce a concise, easy-to-mass produce and easy-to-read assay to measure insecticide susceptibility for mosquito adulticides.

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### INSECTICIDE RESISTANCE STATUS OF THE ASIAN TIGER MOSQUITO IN THE UNITED STATES

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*Aedes albopictus*, the Asian tiger mosquito (ATM), the principal vector of chikungunya, is an introduced invasive species in the US responsible for a significant proportion of service requests to local mosquito control programs. ATM was first detected in the US in 1985 but is now one of the most common pest mosquitoes responsible for many service calls that result in the application of insecticides. However, to date very limited information is available on the insecticide resistance status of US ATM populations. Because of the possible impact of insecticide resistance on present and future ATM control operations we implemented the current studies. Specifically, we focused on the insecticide resistance status of ATM populations from New Jersey, Pennsylvania and Florida. Overall we tested nine populations (5 from NJ and 2 each from PA and FL). We implemented larval and adult bioassays following WHO standard protocols. We chose a range of insecticides representing classes or type of insecticides with different modes of action currently or historically used in the US for mosquito control (organochlorines, organophosphates, pyrethroids, carbamates, insect growth regulators and bioinsecticides). Larval bioassays revealed overall complete susceptibility to most insecticides but we did find some populations with reduced susceptibility to a carbamate. Similarly, most adults tested with WHO tube tests were fully susceptible to the majority of insecticide classes but surprisingly we found evidence of high levels of resistance concentrated in a few populations. To investigate the possible mechanisms involved in resistance such as metabolic-based resistance (Oxidases, Glutathione S-Transferases, and Esterases) and target-site resistance (kdr and ace mutations) we developed both biochemical and DNA based assays. In light of the results we will discuss the efficacy of different insecticide classes used for ATM control, and the resistance, or cross resistance patterns in US ATM that may threaten future control operations.

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### ELECTRONIC DATA FOR INDOOR RESIDUAL SPRAYING (IRS): A PILOT TO CAPTURE, MAP AND MONITOR SPRAY ACTIVITIES IN ZAMBIA

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Indoor residual spraying (IRS) along with long-lasting insecticide treated nets (LLIN) form the mainstay of vector and subsequently malaria control throughout sub-Saharan Africa. Zambia has invested heavily in IRS over the past decade and now boasts 80% target coverage levels in urban/peri-urban settings contributing to a significant reduction in national malaria parasitemia from 22 % in 2006 to 16% in 2010. Historically, spray data is collected on paper, which is then manually aggregated and entered into a spreadsheet for reporting. Multiple spreadsheets are then manually combined to produce the final dataset. This system is labour intensive, prone to errors and limited in scope. To address this issue, an electronic data capture solution (mSPRAY) was developed and piloted in Chibombo district, Zambia, for rapid collection and dissemination of IRS data. IRS operators were individually equipped with a personal digital assistant (PDA) pre-loaded with the mSPRAY software that guides them through collection of all data elements including GPS coordinates for every structure, spray application details, LLIN usage and previous spray history. Validation rules built into the software ensured that only valid data was entered. Supervisors were able to review these data at any stage to increase accuracy. Periodically, datasets were exported for timely reporting to the district / provincial / central level(s). During the season, mSPRAY was able to provide regular feedback on overall performance. As a result it was able to identify a major shortfall in reaching total target structure coverage. Based on this data, operational changes were made to address this issue and coverage was dramatically improved. mSPRAY also identified areas missed during spraying that were originally targeted, again allowing spray teams to revisit these overlooked areas. In short, mSPRAY offers a robust and expanded data collection method allowing fine spatial mapping of spray activities to ensure that IRS applications are as effective and efficient as possible.

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### EVALUATION OF LONG-LASTING ALPHA-CYPERMETHRIN IMPREGNATED NETS IN ATTRACTIVE LETHAL OVITRAPS (ALOT) AGAINST *Aedes aegypti* FOR DENGUE CONTROL IN IQUITOS, PERU

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Dengue is the most important mosquito-borne viral disease in the world, and *Aedes aegypti* is the primary vector in the majority of affected tropical countries. Without an effective vaccine, chemical control of the adult vector is an important weapon in reducing disease impact. Our study was carried out in Iquitos, Peru, an urban city located in the Amazon basin, where dengue epidemics have been reported since 1990, with the largest in terms of morbidity and mortality occurring between 2011-2012. In June 2011, we initiated a large efficacy trial of a novel Attractive Lethal Ovitrap (ALOT) to reduce dengue and its mosquito vector. The ovttraps had 3 components: 1) a black and red colored outer structure that visually attracted mosquitoes; 2) an alpha cypermethrin 0.55% impregnated

long-lasting net that killed adults when they rested inside the trap; and 3) a packet containing a larvicide (spinosad) with lyophilized bacteria (attractant) applied to water in the base of the trap. We evaluated the efficacy of the net component (DuraNet™) of the ALOT traps between June 2011 and April 2012. We randomly selected twenty nets from approximately 7,000 traps deployed in approximately 2,800 houses and evaluated them in duplicate under lab conditions using two strains of *Ae. aegypti*: New Orleans and Iquitos. Nets without insecticide were used as controls. Mortality (24 hr %) ranged from 72-100% in the Iquitos field-derived strain and 99-100% in the New Orleans susceptible control strain. The results indicate that the net component of ALOT traps is maintained over an 8-month period under field conditions.

## 729

### MULTICENTRE STUDIES OF INSECTICIDE-TREATED DURABLE LINING IN AFRICA AND SOUTHEAST ASIA: ENTOMOLOGICAL EFFICACY AND HOUSEHOLD ACCEPTABILITY DURING ONE YEAR OF FIELD USE

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Indoor residual spraying (IRS) is a primary method of malaria vector control but its potential impact is constrained by several inherent limitations: spraying must be repeated when insecticide residues decay, householders can tire of the annual imposition and campaign costs are recurrent. Durable Lining (DL) is a deltamethrin-impregnated polyethylene sheeting material that can be used to cover walls and ceilings of domestic habitations that would normally be sprayed with insecticide. It can be considered a form of long-lasting IRS in which insecticide is released gradually from an aesthetically attractive wall covering. The operational success of DL will be contingent on attaining a high level of user acceptability as households need to maintain correctly installed materials on their walls for a number of years. A one year multicentre trial was conducted in 480 households from seven malaria endemic areas (Angola, Equatorial Guinea, Ghana, Mali, Nigeria, South Africa and Vietnam) representing the largest field evaluation of DL to date. At each site the durability, bioefficacy and household acceptability of DL was assessed compared to conventional IRS and other long-lasting insecticide-treated products. Over the year, the DL demonstrated little to no decline in bioefficacy, which was supported by a small loss of insecticide content. The majority of participants reported reductions in mosquito density (93%) and biting (82%), but no adverse changes to their indoor environment (83%). The DL was well received, more so in rural than in urban houses, because of its perceived efficacy and aesthetic value. When offered a choice of vector control product at the end of trial, DL always emerged as the most popular intervention regardless of the earlier household allocation. These results suggest that DL could overcome many of the user constraints associated with spray campaigns and has the potential to become a viable, long-lasting alternative to IRS in malaria endemic areas.

### SMALL-MOLECULE INHIBITION OF 'KIDNEY' FUNCTION IN MOSQUITOES

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About half of the world's population is at risk of contracting malaria, which kills 1 million individuals each year. Increased resistance in the malaria vector mosquito *Anopheles gambiae* is threatening the use of common insecticides such as pyrethroids. The development of novel insecticides with new modes of action is therefore essential for controlling mosquito populations and limiting malaria transmission. Malpighian tubules, the kidneys of mosquitoes, are essential for the survival of mosquitoes after they consume a blood meal, because they mediate the excretion of excess salts and water that are ingested. Our previous physiological studies of isolated Malpighian tubules have shown that barium-sensitive potassium channels are critical to the excretion of salt and water by this epithelium. Recently, we have cloned a barium-sensitive, inward rectifier potassium (Kir) from the Malpighian tubules of the yellow fever vector *Aedes aegypti* (AeKir1). We hypothesize that chemical disruption of AeKir1 will inhibit Malpighian tubule function and make mosquitoes more susceptible to the physiological stresses associated with blood feeding. Here, we report the discovery of a small-molecule antagonist termed VUXXX that inhibits heterologously expressed AeKir1 with a 50% inhibitory concentration of 10 µM, providing a tool compound for exploring the physiology and viability of AeKir1 as an insecticide target. Consistent with AeKir1 being essential for urine formation, VUXXX at a concentration of 5-10 µM inhibits fluid secretion in isolated Malpighian tubule assays. Medicinal chemistry is being used in an effort to increase the potency and selectivity of VUXXX for AeKir1 over mammalian Kir channels. In an effort to identify other inhibitors of AeKir1, we have developed and implemented a fluorescent-based high-throughput screening (HTS) assay to support a drug discovery campaign for AeKir1. This study is the first to target the mosquito renal system for the development of novel insecticides. Funding is provided by a grant from the Foundation for the NIH, VCTR program.

## 731

### PREVENTING THE SPREAD OF THE VIRUS VECTOR *Aedes albopictus* TO THE AUSTRALIAN MAINLAND: A CAMPAIGN OF SUPPRESSION IN THE TORRES STRAIT ISLANDS

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Dengue is the leading arboviral health issue in Australia. There are hundreds of imported cases annually, and local transmission, mediated by *Aedes aegypti*, has resulted in multiple outbreaks in northeastern Queensland and the Torres Strait Islands (36 outbreaks and 2,365 cases since 2000). The region's Dengue Action Response Team (DART) is generally successful at constraining outbreaks and eliminating the virus. This is achieved through active case management and highly targeted vector control measures. Australia now faces a further threat from another dengue vector; *Ae. albopictus*. This is the world's most invasive mosquito. Its presence would dramatically complicate the epidemiology and control of dengue transmission in the region. Its climatic tolerances also predict that it would establish over a far greater area than *Ae. aegypti*. It represents a considerable public health risk and a public nuisance for Australia. It established on the Torres Strait Islands in 2005 but is yet to reach the mainland despite considerable traffic between these areas. This is largely because the DART conducts a campaign of vector suppression that focuses on the hubs of the major transport routes to the mainland.

*Ae. albopictus* is adapted to a more sylvan habitat than *Ae. aegypti*. Its management has therefore necessitated the careful development and adaptation of vector control tools and monitoring methods by the DART and the construction of an evidence base that demonstrates their utility. The campaign has been successful at reducing the *Ae. albopictus* population on the target islands to extremely low levels. In contrast, neighbouring untreated islands have experienced dramatically increased numbers. On the targeted islands, *Ae. aegypti* remains the commoner of the two species. Elsewhere it has been completely displaced by *Ae. albopictus*. This is the result of the differential susceptibility of these species to a program specifically targeted against *Ae. albopictus*. To date, the activities of the DART have been successful at protecting Australia from a mainland incursion. The sustainability of the approach is, however, unclear. A number of other tools for both vector control and disease mitigation are being explored.

### 732

#### POTENTIAL FOR PYRIPROXYFEN TO STERILIZE *ANOPHELES ARABIENSIS* MOSQUITOES

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Achieving malaria elimination will require new ways to control insecticide resistant and outdoor biting/resting mosquitoes. The most abundant malaria vector in much of East Africa is *Anopheles arabiensis* which shows exophilic and exophagic behaviour and has developed pyrethroid resistance in some areas. The aim of this study was to determine whether Pyriproxyfen (PPF), a juvenile hormone regulator, could be used to sterilise those mosquitoes not successfully targeted with current control methods such as insecticide treated bed nets and indoor residual spraying. The bottle assay was used to expose mosquitoes to PPF at a rate of 0.003g AI/m<sup>2</sup>, with control mosquitoes subjected to the same conditions without the PPF. Differing levels of sterilisation were observed varying from none, to complete, depending on the time of blood feeding in relation to PPF exposure. Mosquitoes with fully developed eggs at the time of exposure, or fed post exposure showed little reduction in fecundity and fertility. Mosquitoes fed 1 day prior to, or during exposure, showed near complete reductions in fecundity and of the small number of eggs laid, none hatched into larvae. These results therefore open up new avenues for control measures for example in combining PPF into pyrethroid treated bed nets as a method of controlling resistant mosquitoes, or as a lure and sterilise technique for outdoor biting mosquitoes.

### 733

#### EXPANDING THE FOOTPRINT OF *Aedes albopictus* IN BELIZE, CENTRAL AMERICA

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The Asian tiger mosquito, *Aedes albopictus* (Skuse), is an aggressive daytime-biting mosquito closely associated with human activity and a vector of several viral diseases including dengue, chikungunya and yellow fever. Native to Southeast Asia, its status as an invasive species is well known. Against the backdrop of increased reporting of dengue fever cases, the first human-landing report of adult *Ae. albopictus* in Belize occurred in 2009 at Benque Viejo del Carmen, a city along the Guatemalan border in the Cayo District. We report here the first record of *Ae. albopictus* from the northern Orange Walk (OW) District, Belize and the insecticide resistance status of this population to malathion (Fyfanon®), the standard Belize MoH adult dengue vector control intervention.

### 734

#### TOWARDS A PUSH-PULL STRATEGY FOR MALARIA VECTOR CONTROL: OUTDOOR MOSQUITO TRAP DYNAMICS IN NORTHERN BELIZE, CENTRAL AMERICA

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Current vector control tools are fast becoming inadequate to control malaria for a number of reasons, including insecticide resistance. With the intent to eliminate malaria, novel approaches are required. Our team is evaluating the use of spatial repellents and outdoor mosquito traps in a combined push-pull strategy to reduce the probability of human-vector contact. This study reports on the first of a series of experiments in Belize, Central America intended to develop and evaluate this integrated approach for malaria prevention. Two commercially available outdoor mosquito traps, the CDC miniature light trap and BioGents-Sentinel™ mosquito trap, were evaluated in a series of all-night experimental hut studies to compare trap densities of natural *Anopheles spp.* mosquitoes. Results indicate that CDC traps captured greater mosquito densities per hour than BG-Sentinel™ traps for three prevalent local malaria vectors: *An. vestitipennis*, *An. albimanus* and *An. punctimacula*. With the exception of *An. punctimacula*, mosquito entry patterns into experimental huts (i.e. peak entry time and overall numbers) were similar regardless of which trap was deployed. In a subsequent study, CDC traps captured an average of 80.6 *Anopheles* mosquitoes per night, but there was no significant impact on the number of *Anopheles* mosquitoes entering the huts compared to no-treatment controls. These findings suggest that while outdoor traps readily remove vectors from the peridomestic area, the impact on overall numbers of host-seeking mosquitoes was negligible. Moreover, those anophelines not captured were not deterred from entering huts occupied by human hosts. Future studies will quantify the effects of integrating indoor spatial repellent treatments on mosquito entry and on outdoor trap dynamics to define potential increased efficacy of an integrated push-pull approach.

### 735

#### DEVELOPMENTAL PLASTICITY OF CONTAINER MOSQUITOES IN RESPONSE TO VARIABLE ENVIRONMENTAL CONDITIONS AND DENSITY

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Few studies have sought to determine relative importance of multiple environmental factors on developmental life-history traits of mosquitoes, despite the demonstrated effects of many single factors on individual variation. Understanding the drivers of development is of critical epidemiological importance in mosquito vectors in order to facilitate accurate prediction of populations in response to rapid, global climate change. We analyze the relative importance of multiple environmental conditions on the phenotype of development rate, assess possible interactions between environmental factors, and provide a measure of the phenotypic plasticity across gradients of environmental conditions in the yellow fever mosquito, *Aedes aegypti*. A meta-analysis of the empirical estimates of developmental studies over 100 years of research on *Ae. aegypti* allows us to search for broad phenotypic patterns over time and space. The meta-analysis reveals significant heterogeneity in developmental timing between studies. The environmental factor of temperature significantly explains this bulk of this heterogeneity in the literature. The meta-analysis is limited by literature bias because most studies have focused solely on temperature, and conduct experiments under limited ranges of intraspecific rearing density or food availability. As a result, other environmental factors known to potentially



influence development rate could not be detected. To address this, we experimentally estimate the norms of reaction in development rate of *Ae. aegypti* in controlled environmental chambers over wide gradients of multiple environmental conditions including temperature, food availability, and intraspecific rearing density. Results demonstrate that *Ae. aegypti* development rates are highly plastic across varying environmental conditions spanning poor to high resource quality. In contrast to the results of the meta-analysis, this developmental rate variation cannot be explained by temperature alone. Rather, the quality of the environment influences rate of development and is determined by multiple factors and significant, complex interactions between these factors.

## 736

### JAPANESE ENCEPHALITIS VECTOR MODEL DEVELOPMENT: AN AFHSC-GEIS NETWORK EFFORT

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Established in 1997 by Presidential Directive NSTC-7, the Department of Defense (DoD) Global Emerging Infections Surveillance and Response System (GEIS) was incorporated as a Division of the Armed Forces Health Surveillance Center (AFHSC) in 2008. The AFHSC-GEIS partner network includes five core DoD overseas laboratories and over 30 other partner institutions within the US and abroad. GEIS partners work in conjunction with host nations to conduct global disease surveillance that supports US Force Health Protection as well as local health interests. AFHSC-GEIS strives to maintain a centrally coordinated network in which their partners not only report to GEIS, but collaborate with one another to create stronger products that enhance DoD and host nation health systems for improving disease risk and threat reduction strategies. This has been accomplished in part by sustaining central laboratories that supply partners with reagents, laboratory and technical support, as well as by instituting internationally harmonized efforts in the fields of influenza and malaria surveillance. This collaboration has also been achieved in the development of disease risk and prediction models. One of the most salient examples is a distribution model for the primary Japanese encephalitis (JE) mosquito vector, *Culex tritaeniorhynchus*, developed at Uniformed Services University of the Health Sciences (USUHS). This ecological niche (EM) model was created by experts at USUHS using mosquito location data obtained by GEIS partners in the Republic of Korea (ROK) and Thailand as well as environmental data obtained through GEIS partner researchers at NASA. The original EM model in 2009 was limited to the ROK, but has since been refined and expanded to Southeast Asia and is now available to the general public via VectorMap ([www.vectormap.org](http://www.vectormap.org)), another GEIS supported project. This model has been used by the Joint Preventive Medicine Policy Group to help inform JE vaccination policy for US service members, demonstrating the relevance of AFHSC-GEIS products to the US and to global health interests.

## 737

### DEMONSTRATION OF A PUSH-PULL STRATEGY INTEGRATING THE AUTO-DISSEMINATION OF A LETHAL AGENT FOR AEADES AEGYPTI CONTROL IN IQUITOS, PERU

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Dengue is one of the most important viral diseases in the world. Mosquito control plays an important role in the prevention of infection, due to the lack of an effective vaccine and treatment. A repellent focused push-pull strategy to reduce *Aedes aegypti* inside homes is currently in the proof-of-concept development phase and being evaluated under field conditions. The strategy is based on the use of spatial repellents inside homes (the push component) to discourage adult mosquitoes from entering the treated space and an attractant trap (the pull component) placed outdoors to remove repelled vectors from the peridomestic environment. Combined, the goal is to reduce human-vector contact and the probability of virus transmission. However, it is theorized that the impact of the trap (pull) component could be enhanced by the introduction of a lethal agent (pyriproxyfen) that is auto-disseminated to larval sites by the mosquitoes that have been lured and captured. This push-pull/contaminate-release approach could have a wider effect on vector populations over time. The objective of the current study was to demonstrate under experimental field conditions the feasibility and efficacy of the approach. We report on the use of a mark-release-recapture design to quantify the reduction of *Ae. aegypti* entry into experimental huts using a spatial repellent and the BioGents-Sentinel™ mosquito trap with subsequent counts of mock lethal agent auto-dissemination events in sentinel ovitraps. This information will guide future optimization of the push-pull strategy in preparation for pilot field trials in local homes.

## 738

### THE PREFERENCE OF ANOPHELES FUNESTUS TO EITHER FEED INDOOR OR OUTDOOR CHANGED WITH TIME AND VECTOR CONTROL INTERVENTIONS IN LUANGWA VALLEY, SOUTHEAST ZAMBIA

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Both personal and community-level impacts of indoor residual spraying (IRS) and long lasting insecticidal nets (LLINs) are mainly dependent upon mosquitoes entering houses, and thus understanding the behaviour of malaria vectors in relation to vector control interventions is fundamental; and is highly variable across the different vector species. The behaviour of *Anopheles funestus*, the primary vector of malaria in Luangwa valley, South east Zambia has been monitored for three consecutive years (2010 - 2012) to evaluate the changes with the ongoing vector control interventions in the area. The main vector control intervention in the first two years was LLINs, but this was complemented with IRS in the third year. Mosquitoes were collected by human landing catches in blocks of houses during the main transmission seasons. At the baseline *An. funestus* equally predisposed to bite indoor or outdoor: the proportions [95% confidence interval] caught indoors were 0.586 [0.303, 0.821]. Increase in endophagy were observed in 2011 and 2012, and the proportions [95% confidence interval] caught indoors were increased to 0.763[0.599, 0.874] and 0.856 [0.437, 0.978], respectively. The mean biting rate was 9.38 bites per person per night at the baseline and this was increased to 17.08 in the second year, and followed by very high reduction to 1.33 bites per

person per night in the third year following the implementation of IRS with pyrimiphos-methyl. The peak biting time was in the late hours of the night, well after people go to sleep and unchanged during the study period. The results are discussed in the context of increased resistance to pyrethroid insecticides and introduction of IRS with pyrimiphos-methyl for malaria vector control in the area.

### 739

#### ENVIRONMENTAL DETERMINANTS OF RESTING AND FEEDING BEHAVIOR IN MALARIA VECTORS AND ITS IMPLICATIONS FOR MALARIA TRANSMISSION CONTROL

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In recent years the vector species *Anopheles arabiensis* is increasingly responsible for malaria transmission in Africa and reports indicate that in areas of high insecticide treated net coverage, *An. arabiensis* has become the dominant vector species. Unlike its sibling species *An. gambiae* s.s., *An. arabiensis* exhibits much more variable feeding and resting behaviours which will have a strong influence on their transmission capacity and susceptibility to domestic-based vector control measures. This study aims to identify the impact of ecological factors and control measures on the epidemiologically relevant feeding and resting behaviours of *An. arabiensis* within an area of intense malaria transmission, the Kilombero Valley of Tanzania. Here, longitudinal surveillance of vector behaviour has been initiated in four villages where pilot investigations have shown *An. arabiensis* behaviour to vary. Investigation of seasonal variation in the host-seeking (time, and location) and resting behaviour (indoor versus outdoor) of *An. arabiensis* is being conducted throughout the rainy and the dry season. The relationships between vegetation characteristics, climate parameters as well as the local availability of alternative hosts such as livestock numbers to spatial and temporal variation in mosquito behaviours was tested. Anthropogenic factors including housing type, number of household members and bed nets were also recorded. Ecological niche modelling mixed effects modelling and other multivariate statistical approaches were used to identify the contribution of these factors to the host-seeking time and location of *An. arabiensis*. Results showed *An. arabiensis* behavioural variation over a small geographical range associated with environmental variables. Overall *An. arabiensis* was found to prefer to bite early and outdoors at high frequency; a strategy which allows them to minimize contact with Insecticide Treated Nets. The vector was also found predominantly resting outdoors. We assessed how these behavioural characteristics impact on current control measures and affect the potential direction for future control measures. Our findings can help to inform decisions about large scale vector control strategies and their expected impact on target vector populations in different ecological settings.

### 740

#### ALTERNATIVE MOSQUITO VECTOR COLLECTION METHODS IN A SUDAN SAVANNAH AREA OF MALI THAT RECEIVED FIVE MDA ROUNDS FOR LYMPHATIC FILARIASIS ELIMINATION

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To assess the efficacy of two new vector collection methods and their ability to measure vector survival rate, the BG sentinel trap (BGST) and the Ifakara tent trap type C (ITTC) were compared to the more routine Human Landing Catch (HLC) method in 2 villages in Sikasso District in southern Mali that had received 5 annual MDA with albendazole plus

ivermectin. Mosquitoes were collected monthly at three sites in each village. The sites in each village were at least 100 meters apart, and the three methods were implemented concomitantly at each site with one BGST trap, one ITTC trap and one HLC unit (one room with two collectors-one inside and the other outside the room). *Culex* spp. were the most common species collected regardless of the method used. Of the 4,500 mosquitoes collected from July to December 2011, 2,755 (61.2%) were *Culex* spp and 1,745 (38.8%) were *Anopheles* (*Anopheles gambiae* s.l and *An. funestus*). The yield of anopheles, the vectors of lymphatic filariasis in this region, increased from July to September before decreasing for all collection methods. The total number of anopheles collected by the HLC method was 1,019. This was 34 and 1.5 fold higher than that for the BGST and the ITTC, respectively. Interestingly, there was a significant correlation between the monthly yield of *Anopheles* captured by HLC and the ITTC ( $r=1$ ,  $p=0.003$ ) but not by HLC and the BGST ( $r=0.77$ ,  $p=0.10$ ) in the village (Bougoula) in which 85.6% of the anopheles were collected. In Boundioba, the correlation between the HLC and the ITTC and the BGST yields were not significant ( $r=0.61$ ,  $p=0.19$  and  $r=0.53$ ,  $p=0.27$ , respectively). In Bougoula, the *Anopheles* survival rates varied depending on the collection method: 0.92 for HLC, 0.93 for ITTC and 1 for BGST. In contrast, in Boundioba, the survival rates were similar between the methods (0.95, 0.96 and 0.95, respectively). In conclusion, the ITTC appears to be a good alternative collection method to HLC if the vector density is high. This is becoming important as monitoring mosquito vectors and infections rates become increasingly important for the LF elimination process.

### 741

#### A NEW, EXPOSURE-FREE TRAPPING METHOD TO ASSESS THE BEHAVIOURAL VARIATION IN THE MALARIA VECTOR ANOPHELES ARABIENSIS

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The *Anopheles arabiensis* mosquito has become the dominant vector responsible for malaria transmission in the Kilombero Valley, Tanzania and many other regions of Africa. This species exhibits variable host-seeking and resting behaviour. Consequently a more diverse range of trapping tools is needed to permit surveillance of this vector in the varied indoor and outdoor habitats in which it is active. Historically, the most commonly used means to collect anthropophilic malaria vectors was the Human Landing Catch. However, this method is increasingly considered to present ethical issues, as it involves exposing human 'baits' to potentially infectious mosquito bites. Furthermore, this method is labour intensive and hindered by the inherent variability in the attractiveness and skill of collectors. This method is also unsuitable for trapping of zoophilic mosquitoes. Here we evaluated the use of electrocuting grids placed around protected live bait as an exposure-free alternative to the Human Landing Catch. The method offers a more efficient, representative and ethically permissive way for sampling mosquitoes with different behaviours. The novel method makes use of live hosts, animal or human, but ensures they are protected by a mosquito net preventing exposure. The traps can be placed inside houses as well as outdoors. A simple, portable roof protects the trap from heavy rain. This method was evaluated against commercially available insect zappers and Human Landing Catch over 21 nights in Lupiro Village, Tanzania. Results showed a consistent performance of the electric grid traps which compares favourably with insect zappers and Human Landing Catch. The electric grid trap proves to be an excellent alternative sampling tool for malaria vectors which can be applied in many settings. Its flexibility in terms of bait type and trapping location permitted an exposure-free investigation in to the host-seeking behaviour of *Anopheles arabiensis*.

### THE ROLE OF SPATIAL REPELLENTS IN THE CONTROL OF MOSQUITO BORNE DISEASES

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Spatial repellent consumer products have been shown to effectively reduce man-vector contact. They are highly acceptable by most people hence increasingly being used for personal protection. This opens up a niche that can be exploited in order to attain maximum gains in vector borne disease control. Spatial repellents provide a protective bubble within which mosquitoes cannot access humans thus may be effective against mosquito vectors which are now increasingly biting humans outdoors where they remain unprotected. There is need to determine whether repellency as well as modification of mosquito behavior are likely to contribute towards disease reduction. The aim of this study was to quantify the repellency of different doses of transfluthrin (a pyrethroid with a unique chemical formula allowing it to evaporate at ambient temperature and protecting a given space) against mosquitoes outdoors. We used a novel system in which mosquitoes were allowed to respond to different stimuli. This system also enabled the measurement of mosquito responses to stimuli at different distances outdoors. This allowed us to determine the protective effective distance of different doses of transfluthrin. In addition, we conducted experiments to determine whether exposure to different doses of transfluthrin influenced the feeding propensity of mosquitoes as well as the rate of survival of mosquitoes. Results from this study will include dose-response relationships with the main outcome measures being repellency, reduced blood feeding, feeding propensity and survival rate of mosquitoes exposed to different doses. Hence we will provide evidence of the value and role of repellency and mosquito behavioral modification as a highly effective means for controlling disease especially that which is transmitted outdoors.

### SIMULATIONS SUGGEST THAT RELATIONSHIPS BETWEEN MALARIOLOGICAL INDICES ARE STRONGLY MODIFIED BY SEASONALITY

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Evaluating the effectiveness of malaria control interventions on the basis of their impact on transmission instead of their impact on morbidity and mortality is becoming increasingly important as countries move from malaria control to pre-elimination and elimination scenarios. Data on prevalence and transmission are traditionally obtained through resource-intensive entomological and epidemiological surveys that become difficult as transmission decreases. This work employs mathematical modeling to examine the relationships between malaria indices to allow more easily measured data, such as serological data and routine health systems data on case incidence, to be translated into measures of transmission. Scenarios with different levels of transmission and patterns of seasonality were created for populations with realistic age-distributions. Simulations of these scenarios were run with an ensemble of models of malaria epidemiology and within-host dynamics. The simulation results show malaria indices are statistically correlated, allowing for estimates of the relationships between these indices for different seasonality profiles. From regression results we take data from one malaria index and calculate the expected range of values for annual average entomological inoculation rate (EIR), prevalence, incidence of uncomplicated and severe episodes, and mortality by age group across seasonal patterns. These results allow for a direct comparison of malaria transmission using data collected with different methods on different indices. For example, when interventions reduce transmission to levels where entomological surveys are no longer

feasible, data on incidence can be translated to EIR to measure the effectiveness of the interventions. Although the level of seasonality in transmission is rarely considered in data compilations, modeling results show it can be critically important in determining the relationship between transmission and disease, especially in low transmission areas. Understanding relationships between malaria indices addresses key concerns with the traditional methods of quantifying transmission in areas of differing transmission intensity and sparse data. Although these results still need to be validated, along with seasonal data they can help public health officials detect changes of disease dynamics in a population and plan and assess the impact of malaria control interventions.

### WOLBACHIA INDUCES DENSITY-DEPENDENT INHIBITION TO DENGUE VIRUS IN MOSQUITOES

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Dengue virus is a mosquito-borne pathogen that in recent decades has become a major international public health concern. Increasing problems in pesticide resistance and the lack of drugs and vaccines make it urgent to develop novel strategies for dengue control. *Wolbachia* is a maternally transmitted intracellular symbiotic bacterium that is estimated to infect up to 65% of insect species. The ability of *Wolbachia* to both induce viral interference and spread into mosquito population makes it possible to develop *Wolbachia* as a biological control agent for dengue control. While *Wolbachia* induces resistance to dengue virus in the transinfected *Aedes aegypti* mosquitoes, a similar effect was not observed in *Aedes albopictus*, which naturally carries *Wolbachia* infection but still services as a dengue vector. In order to investigate the mechanism of this lack of *Wolbachia*-mediated viral interference, we used both *Ae. albopictus* cell line (Aa23) and mosquitoes to characterize the impact of *Wolbachia* on dengue infection. A serial of sub-lethal doses of antibiotic treatment was used to partially remove *Wolbachia* in Aa23 cells and generate cell cultures with *Wolbachia* at different densities. We show that there is a strong negative linear correlation between the genome copy of *Wolbachia* and dengue virus with a dengue infection completely removed when *Wolbachia* density reaches a certain level. We then compared *Wolbachia* density between transinfected *Ae. aegypti* and naturally infected *Ae. albopictus*. The results show that *Wolbachia* density in midgut, fatbody and salivary gland of *Ae. albopictus* is 80-, 18-, and 23-fold less than that of *Ae. aegypti*, respectively, while there is no difference in ovary between two mosquitoes. We provide evidence that *Wolbachia* density in somatic tissues of *Ae. albopictus* is too low to induce resistance to dengue virus. Our results will contribute to our understanding the mechanism of *Wolbachia*-mediated pathogen interference and developing novel methods to block disease transmission by mosquitoes carrying native *Wolbachia* infections.

### SPATIAL DYNAMICS OF ABUNDANCE AND WEST NILE VIRUS INFECTION OF MOSQUITOES IN A SUBURBAN NEIGHBORHOOD

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The spatio-temporal pattern of arboviral transmission depends on when and where virus amplification takes place, and whether amplification patterns persist. Interpretation of such metrics is hampered by the imprecision of measurement and by use of biologically incompatible spatial and temporal resolutions. In this analysis, we placed 32 light traps in a neighborhood with a strong history of West Nile virus infection in mosquitoes using a paired sample design. This design ensured that

distances between traps represented a range of possible distances. During two summer seasons, we collected host-searching and gravid *Culex pipiens* mosquitoes, the main vector for West Nile virus in our study area; gravid females collected from 30 gravid traps were tested for virus. Additionally, we have developed a detailed geographic data set for this region, including locations of structures, water bodies and natural areas, and vegetation characteristics. We then used co-kriging and geostatistical modeling, to graph abundance and infection across the study area as a surface taking into account covariates based on the landscape characteristics of the neighborhood. We then tested for spatial autocorrelation at different temporal scales, and grouped the data temporally using periods with similar weather. We compared patterns of infection and abundance at variable temporal scales and in the context of urban structure and investigated the effect of the uncertainty of mosquito infection on the ability for this measure to be used for a robust and reliable vector index. When taking the season as a single unit, we found that mosquito abundance was spatially autocorrelated, and that this autocorrelation persisted over time, both within and between seasons, while mosquito infection exhibited a more spatially independent pattern. A vegetation index improved the model of abundance but not of infection. Overall our approach appears effective in modeling mosquito abundance, but other parameters need to be incorporated for successful modeling of infection.

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### CHARACTERIZATION OF THE ENVIRONMENTAL FACTORS AFFECTING SWARMING IN *ANOPHELES GAMBIAE*

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Recent advances in insect's biotechnology has opened several options for malaria control among which the release of genetically modified mosquito rendered refractory to pathogen infection. Another approach that may be effective is based on sterile male release. However availability of these tools does not necessarily guarantee ultimate success. Field and laboratory studies designed to dissect the mating biology of mosquitoes are needed to provide a foundation for predicting the potential utility of genetic control and that this study is addressing. Swarms were surveyed and collected in Vallée du Kou, Burkina Faso in 2011. A complete map of swarm distribution across the study site was constructed and overall 300 swarms were spotted across the village. Swarms strongly responded to specific man-made markers within the village. The number of swarms/compound varied from 3 to 20 and the distribution did not follow normal expectations and exhibited substantial variations suggesting heterogeneity in ecological factors that account for swarm occurrence between compounds. Analysis of the spatial structure of swarms based on Monte Carlo simulations of random distributions indicated that swarms were clustered. Nearest-neighbor distance between swarms was significantly smaller if swarms were randomly distributed over space and the kernel density estimation (KDE) indicated hotspots where most of the swarms aggregate as a response to specific environmental cues. A multivariate analysis allowed identifying a subset of environmental parameters that best correlate to swarm structures and that includes, the number of swarm markers/surface unit, the exposition of the makers to sunlight, the contrast pattern and the openness of the marker to air circulation. These preliminary results have significant meanings towards the achievement of genetically modified mosquito strategies because they suggest that swarms respond to specific environmental cues, hence can be predicted and manipulated.

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### EVALUATION OF THE EFFECTIVENESS OF ENTOMOLOGICAL SURVEILLANCE AND VECTOR CONTROL FOR PREVENTION OF DENGUE: SYSTEMATIC REVIEWS OF THE EVIDENCE

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The objectives of the EU FP-7 consortium IDAMS (International Research Consortium on Dengue Risk Assessment, Management and Surveillance) include the development of effective, affordable evidence-based early-warning and outbreak response systems for reducing the impact of dengue. Prior to considering options for new models we reviewed the entomological, epidemiological, meteorological, clinical and economic aspects of dengue surveillance, prevention and control. Vector control remains the only method available to prevent dengue today and entomological indices are still the primary measures of impending risk and intervention success despite doubts about effectiveness in both cases. The findings of reviews of the published evidence on the reliability of entomological surveillance and the effectiveness of dengue vector control tools for dengue prevention are reported here. A defined protocol and standardized approach were followed to review the literature systematically, without restriction on year, location or language. All studies were reviewed by abstract against rigorous inclusion and exclusion criteria. In the case of every included report, two independent reviewers extracted the data. Reports that elicited conflicting interpretations were resolved by discussion. The key findings are summarised and the lessons to be learned and implications for design and implementation of more effective future approaches are discussed.

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### AREA-WIDE MANAGEMENT OF THE ASIAN TIGER MOSQUITO, *Aedes albopictus*: LESSONS LEARNED

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*Aedes albopictus*, the Asian tiger mosquito, is the principal vector of chikungunya and a critical vector of dengue viruses. This daytime biting pest is now distributed over much of the eastern quadrant of the continental U.S. all the way north to coastal New York, and often causes the majority of service requests from urban and suburban residents in New Jersey (NJ) and many other states and nations where it has spread. Our objective was to develop an effective strategy for the area-wide control of *Ae. albopictus*, while demonstrating the public health importance and socio-economic benefits of the area-wide control approach. The project was initiated in the spring of 2008 in two counties in NJ and we have tested multiple control and educational interventions, as well as gauged public interest, public participation, and overall costs. This presentation will highlight our major findings regarding the population dynamics and methodology for control of this species in the US.

## HOST SELECTION, DEFENSIVE BEHAVIORS AND FEEDING SUCCESS OF *CULEX QUINQUEFASCIATUS* IN EXPERIMENTAL TRIALS

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Studies describing common blood sources of field collected mosquitoes are inconsistent in their description of the host selection behavior of *Culex quinquefasciatus*. Host selection is an important determinant of pathogen transmission, and this knowledge gap in mosquito behavior is limiting our understanding of vector-host contacts and the importance of reservoir hosts in West Nile virus (WNV) transmission. We conducted host-choice experiments under semi-natural conditions to quantify host feeding preference by *Cx. quinquefasciatus* mosquitoes when presented with an array of common passerine hosts: Northern Cardinals, American Robins, Blue Jays, Brown Thrashers, and Gray Catbirds. The experimental design consisted of: 1) a 1.5m x 0.75m x 0.75m enclosure inside of which two bird cages were placed, 2) 30 recently emerged female *Cx. quinquefasciatus* originating from wild eggs, and 3) an infra-red camera recording system. We performed 12 two-bird choice experiments in which we calculated the feeding index for each potential host and tested the null hypothesis of random host selection. We also quantified the number of defensive behaviors exerted by each bird. The blood sources for the 168 mosquitoes that successfully obtained a bloodmeal were assessed by amplifying a fragment of the 16s ribosomal gene using generalist avian primers, sequencing each amplified fragment, and comparing the fragment to reference sequences. Host selection differed significantly from random, exhibiting the following preference structure: American Robins preferred over Blue Jays and over Northern Cardinals, and Northern Cardinals preferred over Brown Thrashers. The most common types of defensive behaviors were those protecting the feet and head, but the number of defensive behaviors did not differ significantly between hosts. Further experiments are needed to determine the role of these defensive behaviors in host selection and feeding success by vectors. Our results indicate a non-random pattern of host selection by vectors that needs to be considered when modeling WNV transmission.

## INTERACTIVE TOOLS FOR IDENTIFICATION OF MOSQUITO AND SAND FLY VECTORS OF INFECTIOUS DISEASES

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Computerized interactive tools to identify mosquito and sand fly vectors of infectious human diseases were developed for various regions of the world (see Walter Reed Biosystematics Unit/WRBU website, www.wrbu.org). Using LUCID programs, WRBU identification keys for mosquito and sand fly vectors and their associated groups included morphological diagnostic characters primarily of the head, thorax, abdomen, legs and wings. Automontage images of diagnostic characters of various insect body parts were attached to each key. Genus and species pages for selected vectors and related groups were developed, including brief basic taxonomy, distribution, bionomics, medical importance, selected references, and detailed photos of habitus and other morphological parts. World catalogs of mosquitoes and sand flies, with updated taxonomy and hierarchic classification were linked to each key. In addition, comprehensive lists of known and potential vectors, and their associated taxonomic information, were included in the WRBU website. New LUCID identification keys were recently developed, namely: African *Anopheles* adult and larval keys (include 140+ species and groups for adult key; 120+ for larval key); South American Culicine mosquitoes (include vector adult and larval keys of *Aedes*, *Culex*, *Coquillettidia*, *Haemagogus*, *Mansonia*, *Psorophora*,

*Trichprosoption*); South American Phlebotomine Sand flies (include male and female keys of genera, subgenera, and vector species of *Dampomyia*, *Evandromyia*, *Helcocryptomyia*, *Lutzomyia*, *Nyssomyia*, *Pintomyia*, *Psathyromyia*, *Psychodopygus*, *Sciopemyia*, *Trichophoromyia*, *Verrucarum* Group). Diagnostic characters, updated taxonomy and related information of new vector identification tools are noted and discussed.

## SRPN2 DEPLETION REDUCES MOSQUITO FITNESS AND BITING FREQUENCY

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The mosquito's immune system is at the vector-pathogen interface and largely determines susceptibility. One consequence of its manipulation can be the reduction in vectorial capacity. Therefore, the mosquito immune system provides potential targets for novel intervention strategies aimed to reduce vector-borne disease burden. Melanization is a powerful immune response in arthropods that leads to encapsulation and killing of invading pathogens. This process renders some mosquito species partially or completely resistant to infection with pathogens of global public health significance. One of its rate-limiting steps of melanization is the activation of prophenoloxidase (PPO), which is controlled by an extracellular proteinase cascade and serpin inhibitors. The molecular composition of this system is largely unknown in mosquitoes with the exception of *Anopheles gambiae* SRPN2 and CLIPB9, which constitute the first known regulatory unit that controls melanization. If uncontrolled, e.g. by the depletion of the inhibitor SRPN2, melanization can kill adult females late in life, and thus potentially reduce the vectorial capacity of *An. gambiae*. This feature makes PPO activation, which is a rate-limiting step in melanin production, a potential target for novel malaria control strategies. Using life table analyses, we determined the consequences of SRPN2 depletion by RNAi on several demographic growth parameters under standard laboratory settings. Net reproductive rate (Ro) was decreased by 29%, while mean generation time was unaffected. As a consequence, doubling time (Td) was moderately increased by 9%. The negative effect on net reproductive rate is largely attributable to a significant decrease in bloodfeeding propensity. Bloodfeeding propensity and survival were disproportionately reduced in older mosquitoes after the first two gonotrophic cycles. As a consequence, the number of potentially infectious bites is at least reduced by 83%. Taken together, these data suggest that SRPN2 constitutes a viable target for novel malaria intervention strategies.

## SPATIAL DISTRIBUTION, SEASONALITY AND BEHAVIOR OF NOVEL MALARIA VECTORS IN THE WESTERN KENYAN HIGHLANDS

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Results from a light trap study carried out in 2010, presented previously, revealed the presence of previously unidentified mosquito species carrying *Plasmodium falciparum* sporozoites in Kisii district in the western Kenyan highlands, an area prone to epidemics of malaria. The majority of these specimens could not be definitively identified to the species level using the commonly used morphological keys, and sequencing revealed that there were no matching published sequences available at ribosomal ITS2 and