IN VITRO METABOLISM-LINKED HEMOTOXICITY ASSAY: VALIDATION AND APPLICATION OF THE ASSAY TO SCREEN NEW ANALOGS AND UNDERSTAND THE MECHANISM OF HEMOLYTIC TOXICITY OF 8-AMINOQUINOLINE ANTIPARASITICS

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Metabolites generated through cytochrome P450-dependent metabolic reactions are responsible for hemolytic effects of primaquine (PQ) and other 8-aminoquinolines (8-AQs). The hemotoxic response of the metabolites generated in situ could be measured by estimation of accumulation of methemoglobin (mHb), kinetic measurement of increase in oxidative stress, and depletion of reduced glutathione (GSH) in a microsomal metabolism-linked hemotoxicity assay (Ganesan et al, Toxicol Appl Pharmacol. 2009; 241:14-22). The assay was validated with two blinded sets of non-hemolytic and hemolytic drugs. Twelve of twelve clinically reported non-hemolytic drugs tested negative, and eight of nine hemolytic drugs tested positive in this assay, the exception being acetaminophen. 8-AQ analogs have also been evaluated. Several agents that replenish intracellular reduced thiols and/or protect the cells from oxidant injury were tested for mitigation of hemotoxic effects of 8-AQ metabolites. N-acetyl cysteine (NAC) has been reported to produce an antioxidant injury were tested for mitigation of hemotoxic effects of PQ metabolites. MAQ; however, MAQ generated about 3-fold higher oxidative stress, and decrease in oxidative stress. NAC partially attenuate the hemotoxic effects of 5-hydroxyprimaquine (5-HPQ), a potential hemotoxic metabolite. A comparative evaluation of 5-HPQ and 8-N-hydroxy-6-methoxy-aminoquinoline (MAQ) showed differential hemotoxic responses. 5-HPQ produced about a 3-fold higher mHb and more prominent depletion of GSH in G6PD-deficient human RBCs than MAQ; however, MAQ generated about 3-fold higher oxidative stress than 5-HPQ. In view of the structural similarities and oxidant potential of aminophenols (APs) and hydroxylated metabolites of 8-AQs, several AP analogs were evaluated in vitro for their hemolytic effects. The 2-APs generated markedly higher hemotoxic response compared to 4-APs, but 3-APs were non-toxic. 4-Methyl and 4-chloro substitutions potentiated the toxicity, while 4- and 5-nitro substitutions completely attenuated the toxicity of 2-APs. The results suggest possible structure-toxicity-relationships of APs and may be useful in designing new non-hemolytic 8-AQ analogs.

PHENOTYPING PRIMAQUINE METABOLITES IN VITRO BY PRIMARY HUMAN HEPATOCYTES USING UPLC-QTOF-MS WITH STABLE ISOTOPE LABELING

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Primaquine (PQ) is an important antimalarial agent because of its activity against exoerythrocytic forms of Plasmodium spp. However, hemolytic anemia is a dose-limiting side effect of primaquine therapy that limits its widespread use in the clinic. The hemotoxicity is believed to be mediated by metabolites; however, the identity of the toxic species has remained unclear due to their highly reactive nature. The major plasma metabolite identified in humans and animals, carboxyprimaquine (cPQ), appears not to be responsible for this toxicity. Identification of minor metabolites in biological matrices poses a major challenge. Drug candidates labeled with stable isotopes in combination with LC/MS can be used to overcome this problem. This study was undertaken to identify the metabolites using UPLC-QTOF-MS from in vitro incubation of a 1:1 w/w mixture of 13C6-PQ/PQ with primary human hepatocytes. An Acquity UPLC™ BEH Shield RP18 column (100 mm x 2.1 mm I.D., 1.7 µm) was used. The mobile phase consisted of water and acetonitrile, both containing formic acid at a flow rate of 0.25 mL/min with gradient elution. Acquity UPLC was integrated with QTOF-MS to combine the efficiency of separation with high sensitivity, selectivity of detection, and accurate mass. Qualitative metabolite identification was performed using Metabolynx XS software. The lock mass compound was leucine enkephalin (m/z at 556.2771 and 278.1141). UPLC retention time, twin mass peaks with difference m/z can be measured by estimation of accumulation of methemoglobin (mHb), kinetic measurement of increase in oxidative stress, and depletion of reduced glutathione (GSH) in a microsomal metabolism-linked hemotoxicity assay (Ganesan et al, Toxicol Appl Pharmacol. 2009; 241:14-22). The assay was validated in a microsomal metabolism-linked hemotoxicity assay (Ganesan et al, Toxicol Appl Pharmacol. 2009; 241:14-22). The assay was validated with two blinded sets of non-hemolytic and hemolytic drugs. Twelve of twelve clinically reported non-hemolytic drugs tested negative, and eight of nine hemolytic drugs tested positive in this assay, the exception being acetaminophen. 8-AQ analogs have also been evaluated. Several agents that replenish intracellular reduced thiols and/or protect the cells from oxidant injury were tested for mitigation of hemotoxic effects of 8-AQ metabolites. N-acetyl cysteine (NAC) has been reported to produce an antioxidant injury were tested for mitigation of hemotoxic effects of PQ metabolites. MAQ; however, MAQ generated about 3-fold higher oxidative stress, and decrease in oxidative stress. NAC partially attenuate the hemotoxic effects of 5-hydroxyprimaquine (5-HPQ), a potential hemotoxic metabolite. A comparative evaluation of 5-HPQ and 8-N-hydroxy-6-methoxy-aminoquinoline (MAQ) showed differential hemotoxic responses. 5-HPQ produced about a 3-fold higher mHb and more prominent depletion of GSH in G6PD-deficient human RBCs than MAQ; however, MAQ generated about 3-fold higher oxidative stress than 5-HPQ. In view of the structural similarities and oxidant potential of aminophenols (APs) and hydroxylated metabolites of 8-AQs, several AP analogs were evaluated in vitro for their hemolytic effects. The 2-APs generated markedly higher hemotoxic response compared to 4-APs, but 3-APs were non-toxic. 4-Methyl and 4-chloro substitutions potentiated the toxicity, while 4- and 5-nitro substitutions completely attenuated the toxicity of 2-APs. The results suggest possible structure-toxicity-relationships of APs and may be useful in designing new non-hemolytic 8-AQ analogs.

ANTIMALARIAL ACTIVITY OF METHYL JASMONATE AND EFFECT ON LIPID PROFILE OF PLASMODIUM BERGHEI INFECTED MICE

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Efforts at eradicating malaria has not yielded the desired results due to various challenges part of which is due to parasite resistance to commonly used antimalarial drugs. As part of the search for new antimalarial drugs, we screened methyl jasmonate (MJ), a fatty acid derived cyclopentanone and a component of the essential oil from flowers of Jasminium grandiflorum for in vivo activity in mice. In vitro study had indicated potential antimalarial activity of MJ. The Rane test procedure was used to assess the antimalarial activity of MJ. Forty-two BALB/C mice were infected with P. berghei NK65 (1 x 107) and divided into 6 groups. Groups 1, 2, and 3 received 10, 25 and 50 mg/kg body weight of MJ respectively.
Groups 4, 5, 6 and 7 received chloroquine 10mg/kg, arteether 3.2mg/kg, ethanol and normal saline respectively. All treatments were administered daily orally for four consecutive days. Thick and thin blood films were made from each mouse for 7 days and weekly for 28 days, stained with Giemsa stain and examined microscopically for parasitaemia. Twenty four hours after last administration, 3 mice from each group were sacrificed with serum used for liver function test and cholesterol, triglyceride, HDL and LDL determinations. Mean survival time were also documented. Methyl Jasmonates treatment resulted in a dose-dependent reduction in percentage parasitemia relative to control. 50mg/kg of MJ caused 54.4 % decrease in parasitaemia relative to chloroquine 81.3% and arteether 99.5% by Day 3. Mean survival time for 50mg MJ was 22.6 days compared with untreated (10-5 days), chloroquine (31.5 days) and arteether (27.2days). MJ like chloroquine and arteether treatment caused a marked decrease in cholesterole, triglyceride and HDL relative to untreated infected mice. There was a significant decrease in alkaline phosphatase MJ caused significant reduction in parasitaemia in a dose-dependent manner but less effective than chloroquine and arteether. MJ did not affect liver function enzymes and lipid profile adversely.

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NOVEL ANTI-MALARIALS: NAPHTHOThIAZOLIUM SALTS WITH POTENT ACTIVITY AGAINST Plasmodium Falciparum in vitro AND P. BERGhei IN VIVO
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Because of emerging resistance to existing drugs, novel classes of anti-malarial drugs with new mechanisms of action are needed. A large library of compounds was synthesized and designed to accumulate in the digestive vacuole of the malaria parasite and potentially catalyze the breakdown of hemozoin. Eight compounds in the original library were highly active against Plasmodium falciparum in vitro. The two most promising compounds are amphiphilic naphtho-thiazolium salts with amine-bearing side-chains. The most active compounds identified thus far are (1) KSWI-19855 which has an IC50 of 75nM against both chloroquine-sensitive and chloroquine-resistant P. falciparum (strains D10 and Dd2) and (2) KSWI-19854 which has an IC50 of 75nM against chloroquine sensitive P. falciparum (strain D10) and 0.5μM against chloroquine resistant P. falciparum (strain Dd2). In murine in vivo efficacy studies, both KSWI-19854 and KSWI 19855 demonstrate greater than 90% activity against P. berghei at 10mg/kg/day for 4 days. We postulate that these amphiphilic compounds reversibly enter the lipid nanospheres where hemozoin is synthesized inside the parasite food vacuole. Once in the food vacuole, we postulate that they depolymerize hemozoin by reducing the Fe3+ in hemozoin to its Fe2+ oxidation state, thereby breaking the iron carbohydrate bonds holding the crystal structure together. Dose ranging studies and studies on the mechanism of action are on going. This project may lead to the clinical development of a desperately needed new anti-malarial drug.

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DEVELOPMENT OF SECOND GENERATION REVERSED CHLOROQUINE DRUGS
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Drug resistance is now seen against all of the approved antimalarial drugs. While eradication is the ultimate goal, currently there is still a need for new therapies to help those afflicted with this disease. We have previously reported on our ‘Reversed Chloroquine’ (RCQ) compounds, of which our lead candidate is undergoing preclinical testing. Here we present a structure-activity relationship (SAR) study designed to develop a ‘second generation’ candidate, to be ready in the event our primary drug stumbles on the preclinical road. Specifically, these next-generation RCQ molecules are designed to continue to improve the toxicity profile, while maintaining excellent in vitro and in vivo antimalarial activity.

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ANTIMALARIAL ACTIVITY AND TOXICITY OF 5 AND 7-METHYLATED PRIMAQUINE ANALOGS
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Primaquine, an 8-aminoquinoline derivative, is the drug of choice for radical cure of relapsing malaria caused by Plasmodium vivax, and is also used as a causal prophylactic agent against both P. vivax and P. falciparum. Primaquine in combination with clindamycin has also been shown to be effective for prophylaxis and treatment of Pneumocystis carinii pneumonia in AIDS Patients. A serious limitation to widespread use of this class of drugs, however, is that they produce reversible methemoglobinemia and hemolysis in individuals who suffer from hereditary glucose-6-phosphate dehydrogenase deficiency. Imino-quinone formed by oxidation of the 5- or 7-hydroxylated primaquine metabolite has been postulated to be responsible for this toxicity. If this mechanism is indeed involved, then substitution of a methyl group at 5 and/or 7-position in the quinoline ring of PQ can block the formation of the toxic metabolites. We prepared 5-, or 7-methylated, 5,7-dimethylated as well as 5-methoxy-7-methylprimaquine analogs and evaluated them for in vivo antimalarial activity in P. berghei mouse malaria model and in vitro methemoglobin formation in red cells incubated with the compounds in the presence of pooled human liver microsomes. Methyl substitutions at the 5 or 7 positions dramatically reduced the toxicity, but these analogs were also devoid of antimalarial efficacy. However, introduction of a methoxy group at the 5-position of primaquine improved the antimalarial activity but also increased its methemoglobin generating capacity in the in vitro assay. Introduction of 7-methyl group to 5-methoxyprimaquine greatly reduced both activity and toxicity. These results suggest that the blocking of activation of the 5 and 7-positions of the quinone ring by methylation significantly reduces both toxicity and activity. These results will be discussed in light of the impact of other structural modifications that may improve the therapeutic window.

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LEAD OPTIMIZATION OF LIVER STAGE ACTIVE ACRIDONE ANTIMALARIAL
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Drugs targeting liver stage malaria offer many advantages in the prevention and eradication of the disease, but nearly all of the antimalarials currently in use or under development primarily act on blood stage infection. We have previously reported the discovery of a novel antimalarial acridone chemotype that displays efficacy against sporozoite-induced Plasmodium infection in addition to efficacy against the blood stage malaria. Significant improvement was achieved in the lead optimization process, and our latest lead candidate demonstrates potent efficacy in the following system: a) Prevention of in vitro Plasmodium
berghei sporozoite-induced development in human hepatocytes with an IC_{50} value of 2.2 ng/ml, comparable to that of atovaquone; b) Full protection from in vivo P. berghei sporozoite-induced liver stage infection in mice at 40 mg/kg (3X, oral doses); c) Low nanomolar inhibition of in vitro P. falciparum blood stage growth against a panel of multidrug resistant parasites; and d) Curative efficacy after oral administration against patent infection with P. yoelii in an erythrocytic murine model with an ED_{50} value of 1.2 mg/kg (3X), superior to chloroquine in the parallel study. Details of the design, chemistry, structure-activity relationships (SAR), safety, metabolic studies, and mechanism of action will be presented.

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EARLY-STAGE PRECLINICAL DEVELOPMENT OF REVERSED CHLOROQUINE (RCQ) HYBRID DRUGS

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We previously disclosed a class of molecules, termed Reversed Chloroquine compounds (RCQs), comprising a chloroquine (CQ)-like moiety linked to a Reversal Agent (RA) moiety, which reverses chloroquine resistance (CQR) in malaria. Structure-activity relationship (SAR) work has shown that the RCQ design is very flexible. We have constructed a substantial library of RCQ molecules that display in vitro efficacy - even sub-nanomolar IC_{50} values - against both CQR and CQS Plasmodium falciparum. The RCQ molecules have enhanced uptake, relative to CQ, into CQR parasites; they also diminish the activity of CQR-associated PfCRT protein mutants which have the ability to enhance efflux from the parasite’s digestive vacuole. A subset of these drug candidates has been tested in mouse models of malaria, and found to be capable of reducing the parasite burden to below detectable limits - an oral cure. Both cytotoxicity and acute toxicity in mice are favorable, as is Ames evaluation of mutagenicity. SAR was applied to minimize hERG binding by the RCQ structures; an electrocardiogram study in guinea pigs to test for cardiac response shows a comparable response to that of CQ to high intravenous doses. Rat pharmacokinetics demonstrate good and tunable plasma levels and clearance times. A candidate RCQ drug has been selected and is moving through early preclinical studies.

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ENANTIOMERIC RESOLUTION OF 8-AMINOQUINOLINE ANTIMALARIALS

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Primaquine (PQ), an 8-aminoquinoline (8AQ) antimalarial agent, is the most prescribed drug for the treatment of relapsing malaria and is also an effective prophylactic agent against all plasmodia species. The major drawbacks of this drug are its short half-life and reversible methemoglobinemia and hemolysis in glucose-6-phosphate dehydrogenase deficient subjects. Studies during PQ development showed that the 4-amino-1-methylbutyl side chain on the 8-amino is prerequisite for optimum antimalarial activity. However, this side chain contains an asymmetric center, and conventional methods of preparation yield a racemic mixture. 1,4-Naphthoquinone (NQ) currently in clinical development, contains the same side chain and is also being developed as a racemate. Previous studies from our laboratory have shown that enantiomeric resolution of PQ into its individual enantiomers yields two analogs with markedly different efficacy, toxicity, and metabolism profiles. Enantioselective influences on efficacy and toxicity have also been observed with several other 8-AQ analogs with 5-aryloxy or 5-alkoxy substituents and the same 8-amino side chain. We have recently developed evidence that one enantiomer of NPC1161 (an analog with the same side chain) shows a 20-fold increase in efficacy over the other in a mouse causal prophylaxis model, but does not show a commensurate increase in hemolytic potential. In spite of the importance of this issue, it has been difficult to study individual enantiomers or to contemplate their economical development because of the lack of an efficient method to resolve them. A simple and generally applicable procedure has been applied to resolve different classes of 8-aminoquinolines as their phthalimides by fractional crystallization as diastereomeric salts with commercially available chiral organic acids. This procedure can be applied to resolve milligram to kilogram quantities, and affords a viable and economical option for development of new 8-AQ analogs.

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IMIDO-SUBSTITUTED NAPHTHOQUINONES: A NEW CLASS OF POTENTIAL ANTIMALARIALS

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The most dangerous form of human malaria is caused by Plasmodium falciparum, accounting for 80% of infections and 90% of deaths. Persistence of this disease in poorer countries of sub-Saharan Africa, Central and South America, and Asia represents a global crisis. Widespread resistance of P. falciparum to chloroquine and other commonly available antimalarial drugs exacerbates malaria mortality and intensifies the search for new drugs. Atovaquone, a hydroxynaphthoquinone, is effective against multidrug-resistant parasites without in vitro evidence of cross-resistance. However, atovaquone is unsuitable for use as a single agent because of the relatively quick emergence of resistance. Several 1,4-naphthoquinone derivatives originally investigated as antitumor drugs have been found to interact with novel targets, suggesting that this class of drugs may be effective against drug-resistant P. falciparum. For this study, imido-substituted chloro-1,4-naphthoquinone (IMDNQ) analogs have been synthesized and evaluated for antimalarial activity. Our hypothesis is that IMDNQ compounds will affect metabolic pathways distinct from those targeted by existing antimalarials and thus will be less susceptible to existing resistance mechanisms. IMDNQs were screened using a high-throughput next generation malaria SYBR Green I assay. Of eight IMDNQs screened, four had IC_{50} values <1ug/ml. Open chain IMDNQ analogs had higher antimalarial activity than cyclic IMDNQ analogs. Additional IMDNQ compounds, particularly open chain analogs, will be screened and the mechanism of action of lead compounds evaluated using a metabolomics approach. Once affected metabolic pathways are defined, evaluation of their direct target(s) and target:drug interactions will be used to further refine the structure of inhibitory compounds. Lead compounds will be evaluated against both drug-sensitive and -resistant parasites to evaluate their potential effectiveness against drug-resistant parasites.

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PROBING THE ANTIMALARIAL MECHANISM OF ACTION OF 1,2,4-TRIOXOLANES IN PLASMODIUM FALCIPARUM

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Artemisinin-based endoperoxides are highly potent, structurally complex trioxane antimarialials. Although ferrous activation of the endoperoxide bridge is considered key to drug activity, the mechanism of cytotoxicity remains elusive. Evidence supports a pathway whereby following iron
activation, endoperoxides form damaging free radical metabolites that target parasite macromolecules. Using fluorescent artemisinin analogs, we demonstrated endoperoxide-dependant labeling of neutral lipid bodies associated with the digestive vacuole. We proposed that localization of artemisinin metabolites was due to formation of covalent adducts that further initiated oxidative damage to parasite membranes, as measured by a free radical-sensitive BODIPY probe. A recently developed class of synthetic endoperoxides, comprising a 1,2,4-trioxolane flanked by cyclohexane and adamantane rings, show promise as potent and safe antimalarials. Here, we describe our efforts to similarly probe the localization and reactivity of the trioxolanes in Plasmodium falciparum.

We applied trioxolane probes tagged with either an adamantane or cyclohexane dansyl group to living malaria parasites for observation by fluorescent microscopy. Our results show that iron activation results in molecular cleavage of the trioxolane producing an alkylating adamantane radical and a cytoplasmic cyclohexane product. Labeling of neutral lipid bodies by the adamantyl portion of the trioxolanes was similar to that seen with the artemisinin analogs. Our collective findings using fluorescent trioxolanes suggests that endoperoxide-based compounds share a similar mechanism of action in malaria parasites that may involve targeting of neutral lipid bodies.

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A NOVEL CLADE OF EUKARYOTIC RIBONUCLEOTIDE REDUCTASE R2 SUBUNITS IS EXCLUSIVE TO APICOMPLEXAN PARASITES

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Apicomplexans are protist parasites of momentous public health and economic importance. The diseases they cause include malaria, cryptosporidiosis, East Coast fever, babesiosis and toxoplasmosis, and result in millions of deaths and billions of dollars in productivity and property losses each year. Research into new drug targets against these pathogens remains a high priority. Apicomplexan-related diseases may be controlled via inhibition of essential enzymes, provided that these proteins differ significantly in sequence or structure from homologs in their respective hosts. Ribonucleotide reductase (RNR) is one of 57 enzymes prioritized as potential drug targets against Plasmodium. RNR provides the only de novo means of synthesizing deoxyribose nucleotides (dNDPs and dNTPs), the essential precursors for DNA replication and repair. While RNR has long been the target of antibacterial and antiviral therapeutics, targeting this ubiquitous protein to control eukaryotic pathogens may raise toxicity concerns due to its similarity to vertebrate RNR enzymes. The eukaryotic RNR holoenzyme is of the form α2β2, and consists minimally of two large R1 and two small R2 subunits (α, β). We identified a novel clade of R2 subunits, R2_e2, which forms a sister group to the clade containing all eukaryote standard R2 subunits, R2_e1. Evidence suggests that R2_e2 subunits are functional and yet the amino acid sequence similarity between the two types of R2 subunits is <50%. Remarkably, while most eukaryotic genomes encode two standard R2_e1 proteins, apicomplexans encode one R2_e1 and one R2_e2. In fact, R2_e2 subunits have so far only been identified in apicomplexan genomes. Our results suggest that the novel R2 subunit unique to apicomplexans is a promising candidate for chemotherapeutic-induced inhibition, as it differs greatly from all known vertebrate RNRs and hence can potentially be specifically targeted.

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INTERPLAY BETWEEN COPY NUMBER VARIATION AND ANTIFOLATE RESISTANCE IN PLASMODIUM FALCIPARUM

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GTP-cyclohydrolase (gch1) is the first and rate-limiting enzyme in the folate biosynthesis pathway and has been found to exhibit extensive copy number variation in isolates from around the globe in areas with a history of longstanding use of antifolates. Specifically in South East Asia, increased gch1 copy number is associated with increased likelihood of point mutations in dihydrofolate reductase (dhfr) and dihydropteroate synthase (dhps), genes which confer resistance to pyrimethamine and sulfadoxine respectively. One hypothesis for this finding is that an increased gch1 is an adaptive response to compensate for less fit, drug resistant enzymes downstream in the folate pathway. To investigate the effect that gch1 copy number has on antifolate-resistant parasites, we used a plasmid-based overexpression system, in which we can manipulate gch1 copy number and expression levels in cultured parasites. We have implemented this system in multiple genetic backgrounds with different drug resistant profiles. We further tested whether the drug sensitivities of our parasite lines were altered using [3H]-hypoxanthine drug assays. Our results demonstrate that increases in gch1 copy number and expression alter drug resistance phenotypes only in parasites bearing a mutant dhfr. This suggests that gch1 amplification increases dhfr substrate concentrations relative to that of the inhibitor, thereby relieving the parasite of pyrimethamine pressure and rendering our current antifolate treatments inadequate. In addition, we have found that there is not a linear relationship between gch1 copy number and expression levels in both isolates from around the globe and in our manipulated parasite lines which warrants further exploration. A greater understanding of the folate pathway and all the factors that play into the development of drug resistance is key to development of new drugs targeting this pathway and to understanding in general how the parasite can adjust to different drug pressures through both point mutations and copy number variation.

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WANING EFFECTIVENESS OF INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY (IPTp) WITH SULPHADOXINE-PYRIMETHAMINE (SP) IN THE PRESENCE OF HIGH SP RESISTANCE IN MALAWI

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Intermittent preventive treatment in pregnancy (IPTp) with sulphadoxine-pyrimethamine (SP) is recommended by the World Health Organization for the control of malaria in pregnancy in sub-Saharan Africa. Malawi was the first country to introduce IPTp with SP in 1993. Parasite resistance has compromised the efficacy of SP in the case-management of symptomatic children, but SP remained effective for IPTp in many areas of Africa. We conducted an observational study of women delivering in an area with high SP resistance (frequency of quintuple dhps/dhfr mutant haplotype >95%) in Blantyre district, Malawi to study the effect of SP resistance on
the efficacy of IPTp-SP in preventing placental malaria and preterm delivery or low birth weight. Previous in-vivo studies in this area indicated that 50% of asymptomatic parasitaemic HIV-negative primi-secundigravidae (G1+2) who received IPTp-SP were parasitaemic again within 42 days. Between Dec 2009 and Sep 2010, 780 HIV-negative women delivered (418 G1+2 and 362 multi-gravidae [G3+]), of whom 2.4%, 12.7%, 51.2% and 33.7% had received none, 1, 2, or 3 or more doses of IPTp-SP and 66.6% reported using a bednet. Among G1+2, the prevalence of placental malaria detected by histopathology or RDT was similar in each dose group (44%; 36%; 41%; 50% in the 0, 1, 2, 3 dose group respectively). Among G3+ the prevalence was lower among women receiving IPT, but there was no difference with each incremental dose (30%; 13%; 13%; 11%). The frequency of preterm delivery or LBW was similar in all dose groups among G1+2. Molecular analyses for SP resistance-associated mutations in dhps 436, 437, 540 and 581, dhfr 51, 59 and 164 and pfmr1 1466 are ongoing and will be presented. These preliminary results suggest an absence of a beneficial impact of IPTp-SP among G1+2 protected by ITNs in this area with high grade SP resistance and near saturation of the quintuple dhps/dhfr haplotype. This raises concern about the longevity of IPTp-SP in southern Malawi and stresses the need to explore alternative drugs or strategies to replace SP or IPTp.

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MONITORING OF DRUG RESISTANCE AFTER INTERMITTENT PREVENTIVE TREATMENT FOR INFANTS AND CHILDREN (IPT/C) IN SENEGAL

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In 2006, the health authorities of Senegal changed drug policy from sulfadoxine-pyrimethamine (SP)/amodiaquine (AQ) to the Artemisinin Combination Therapy (ACT) AQ/artesunate as first-line treatment against uncomplicated falciparum malaria. This was done due to reports of Plasmodium falciparum widespread resistance to SP and AQ. Currently, SP is still used for intermittent preventive treatment (IPT) as a method for reducing malaria morbidity and mortality and is being used in pregnant women (IPTp), infants (IPTi) and is being studied for children (IPTc). This study was undertaken to examine the impact SP use for IPTi and IPTc on the frequency of SP-resistant related haplotypes in the Plasmodium falciparum genes, Pfdrfr and Pfdrfs. Samples were collected during a cross sectional survey in 2010 involving children under five years old living in three health districts located in the Southern Senegal where malaria transmission is high. Overall, 257 samples were P. falciparum positive. Among them, 176 individuals had received SP two years ago through IPTi in two of the districts while 81 did not. All positive samples were analyzed to determine the frequency of SP-resistance related haplotypes in Pfdrfr and Pfdrfs based on results obtained by nested PCR followed by sequence-specific oligonucleotide probe (SSOP)-ELISA. The triple mutant Pfdrfr CIRNI haplotype dominated in both groups (IPTi+ [58%] and IPTi- [50%]). The double mutant Pfdrfr CNRNI haplotype was also found with a frequency less than 5% in both groups. For Pfdrfs, the wild type haplotype SAKAA dominated the control group with 28% (23/81) against 15% (26/176) with a significant difference (p=0.036). The double mutant Pfdrfs haplotypes SGEEA and AGKAS were found in our study with a frequency less than 5% in both groups. The single mutant SGKAA haplotype was more frequent in IPTi+ group (30%) than in IPT- group (5%) the difference is not significant (p=6x10^-6). In conclusion, the present study indicates that using SP for IPTi does not select resistant parasites when follow up is performed long term. Base on WHO recommendation, SP can still be use as IPTi in Senegal because of the very low frequency of Pfdrfs haplotype SGEEA (<5%).

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THE RETURN OF WIDESPREAD CHLOROQUINE SENSITIVE PLASMODIUM FALCIPARUM TO MALAWI

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Since chloroquine-resistant falciparum malaria became pervasive in Africa, the reemergence of predominantly chloroquine-sensitive parasite populations has been documented in Blantyre, Malawi, an urban center in Eastern Africa. This resurgence of sensitive parasites followed a change in national treatment policy from chloroquine to sulfadoxine-pyrimethamine in 1993, and treatment efficacy of 99% was demonstrated in 2005. Studies in other areas of Malawi report varying results on resistance levels outside of this population center. This study evaluated the prevalence of chloroquine drug resistance using a marker in the Plasmodium falciparum chloroquine resistance transporter (pfcrt) gene throughout the country, including rural areas and districts bordering countries where chloroquine use persisted much later than 1993. Dried blood spots were collected from children aged five years or less using two-stage cluster sampling in eight districts across Malawi in 2009. Samples with P. falciparum parasitemia on microscopy underwent PCR amplification and pyrosequencing of the pfcrt gene 76 amino acid region to determine chloroquine resistance status. Of 7145 samples collected, 1168 were found to have parasitemia by light microscopy. Of 696 with sufficient DNA for sequencing only 2 were found to have the chloroquine resistance genotype. This translates to an overall proportion of infections with detectable resistance of 0.003 (95% CI: -0.001, 0.007) and a proportion of 0.167 (95% CI: -0.262, 0.595) in Karonga and 0.007 (95% CI: -0.007, 0.020) in Mwanza, the two districts where resistant samples were found. Sampling over a wide geographic region of Malawi, including higher risk sites for ongoing resistance such as border areas indicates that chloroquine-susceptible malaria now predominates the parasite population in this country. A very small subpopulation of resistant parasite nevertheless appears to persist within this population, suggesting that resumption of chloroquine use might be quickly followed by selection and increasing prevalence of chloroquine-resistant parasites.

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A NETWORK-BASED APPROACH TO PROBING THE METABOLIC PATHWAYS OF PLASMODIUM FALCIPARUM

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There is a growing demand for high resolution data to quantify and characterize the enzymic and metabolic status of the human malaria parasite, Plasmodium falciparum. The resolution of a metabolic network offers insights into the life cycle and pathophysiology of the parasite. Since metabolites are the ultimate cellular readout, investigating the
global metabolic flux regulation of an organism is generally more informative than measuring mRNA levels. Our approach is enhanced by the incorporation of network theory and graphs that model the interconnected and sequential conversions of compounds in metabolic pathways. The profile of individual metabolite levels inherited in progeny of a genetic cross can serve as a phenotype to uncover genetic factors underpinning parasite physiology using quantitative trait loci (QTL) mapping. We extracted metabolites from the parents and progeny of HB3 × Dd2 and 7G8 × GB4 genetic crosses of *P. falciparum* at three erythrocytic cell cycle stages and constructed a metabolic network using Pearson’s correlation of metabolite levels obtained from LC-MS. Individual mass signatures, the vast majority still unidentified, map to all chromosomes in the genome in an asymmetrical manner such that a few loci influence the levels of many compounds while other loci affect none. Our network approach does not rely on QTL; however, the network modularity of clustering patterns of compounds can be used to evaluate the significance of QTL. We investigate whether co-mapping compounds from QTL hotspot regions also cluster together in the network. Finally, the network provides an interpretive framework for the prioritization of these unknown compounds by clustering metabolites involved in specific pathways and by leveraging information about the known metabolites. These studies establish a framework to construct and analyze the metabolite network in *P. falciparum*, and will ultimately provide useful insights about antimalarial drug resistance and prospective targets.

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**GAMETOCYTE CLEARANCE DYNAMICS FOLLOWING ORAL ARTESUNATE TREATMENT OF UNCOMPlicated FALCIPARUM Malaria in Mali, West Africa**

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Artemisinin-based combination therapies (ACTs) reduce *Plasmodium falciparum* gametocyte carriage, but their true effect on gametocytes and on transmission potential is not fully understood. A better understanding of gametocyte dynamics in vivo in the presence of artemisinin is needed. One hundred children aged 1-10 years presenting with uncomplicated *falciparum* malaria to a sentinel site clinic in Bougoula-Hameau, Mali, were treated with seven days of directly-observed oral artesunate therapy from December 2010 to February, 2011. Thick and thin blood smears were prepared and read every 8 hours until three consecutive slides were negative for asexual *falciparum* parasites. Gametocytes were quantified by two trained microscopists using standard WHO procedures. Gametocyte carriage and density were compared at 0, 1, 2, 3, 7, 14, 21 and 28 days after treatment initiation using the chi-square test and the student’s t-test, respectively. Of 92 children in the final analysis, 21 (22.83%) were gametocyte carriers at the time of treatment initiation. The proportion of gametocyte carriers was unchanged at the end of treatment (day 7, 23.91%, p=1.0) and did not significantly decline until day 21 of follow-up (6.52%, p=0.003). The mean gametocyte density at inclusion, 11.78 gametocytes/µl, also remained unchanged at the end of treatment (13.25 gametocytes/µl, p > 0.05) and only dropped significantly at day 28 of follow-up (0.62 gametocytes/µl, p=0.01). Among carriers at inclusion, the median clearance time was 14 days. Among non-carriers, carriers by day 21 of follow-up. Artesunate decreased gametocyte carriage and gametocyte density by the end of the 28-day follow up. However, artesunate did not prevent the maturation of young gametocytes to circulating stage V, as evaluated by standard microscopy. More sensitive gametocyte detection methods may better characterize these dynamics. Further work is needed to determine the role sequestered gametocytes may play in the persistence of peripheral gametocytemia after artemisinin-based treatment initiation.

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**RESEARCH CAPACITY DEVELOPMENT FROM SCRATCH: THE EXPERIENCE OF THE WEST AFRICAN NETWORK FOR CLINICAL STUDIES OF ANTIMALARIAL DRUGS (WANECAM)**

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Malaria remains a major public health problem in much of sub-Saharan Africa. Yet there is little data on the epidemiology, transmission and drug resistance in many areas of the Continent. To address these issues in Guinea, West Africa, we are building human capacity, infrastructure and the regulatory frame work necessary for conducting state of the art clinical research. A TDR initiative of 1997 selected a young Guinean Scientists with strong potential for research. The scientist received nearly 8 years of training in the laboratory and in the field in France and in Mali leading to a MSc and a PhD degrees in Parasitology. He was then invited to join the ECDCP funded WANECAM project. A site assessment visit by senior members of the Network helped in streamlining the needs in human capacity, infrastructure and regulatory environment. A team of 8 young scientists with little or no experience in research was recruited. The team received intensive short-term training in GCP, ethics, computer skills and clinical studies. Training included short-term workshops both in Guinea and abroad, the posting of experienced Malian scientists in Guinea for extended periods and short visits by experienced senior staff from the other network members. Two students were enrolled for MSc training in Burkina Faso and in Mali. Two 4-weel drive vehicles were purchased. A vacant building was obtained from the Government of Guinea and refurbished into a brand new polyvalent laboratory. As a result, the first malaria entomology survey was conducted. A prospective longitudinal study on references ranges of biological parameters, age specific incidence and drug resistance is underway. A solid and emerging malaria research team is now in the building in Guinea. This experience underlines that capacity development in developing countries is a long-term investment on the scientists, the environment, and the physical infrastructure.

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**EFFICACY OF ARTEMETHER-LUMEFANTRINE AND ARTESUNATE-AMODIAQUINE FOR THE TREATMENT OF UNCOMPlicated PLASMODIUM FALCIPARUM INFECTION IN TANZANIA**

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Following the development of drug resistance to anti-malarial first line treatment of uncomplicated malaria with sulfadoxine-pyrimethamine (SP) by *Plasmodium falciparum* in mainland Tanzania the ministry of health and social welfare (MOHSW) introduced artemisinin combination therapy (ACT) with artemether-lumefantrine (ALu) as first line treatment for the treatment of uncomplicated *falciparum* malaria in 2006. There
is growing evidence suggesting that malaria cases over the past three years and entomological inoculation rates (EIR) that are currently monitored in most parts of Tanzania are declining. Despite good malaria control achievements, there is a threat of ACT drug resistance. Due to recent report on the emerging drug resistance to ACT along the Thai-Cambodia border it is critical to our region to monitor the spread of drug resistance to ACT. We set up to conduct an invivo monitoring study at four country-wide representative National Malaria Control Programme (NMCP)’s sentinel sites in May-August 2011 to assess efficacy of ALu and amodiaquine-artesunate both anti-malaria first line in Mainland Tanzania and Zanzibar respectively. The study sites are Mlimba, Mkuzi, Kibaha, and Muheza in the mainland Tanzania. Participants are febrile patients aged 6-59 months presenting at the health facility to be followed up during 28 days to elicit treatment performance. Results of this study will be out by the time of American Society of Tropical Medicine and Hygiene conference in November 2011. We will elucidate the occurrence of drug resistance by PCR using msp1 and glurp. As some of the current molecular genotyping malaria tools are based on SP which is also used for chemoprophylaxis (IPTp or IPTi) we will also generate data on molecular markers (dhfr and dhps) for SP resistance. This analysis will assist to monitor the evolution, spread and intensification of ACT and SP resistance. Results from this study will be used to assist the MOHSW to assess the current national treatment guidelines for uncomplicated. *Falciparum* malaria.

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**EFFICACY OF FIXED-DOSE COMBINATION ARTESUNATE-AMODIAQUINE VERSUS ARTEMETHER-LUMEFANTRINE FOR UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN CHILDREN UNDER FIVE: A RANDOMIZED NON INFEIORITY TRIAL IN DEMOCRATIC REPUBLIC OF CONGO**

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Until now, only a limited number of studies have been published in Central Africa measuring the efficacy of artemisinin combination therapies (ACTs) since their introduction. The Democratic Republic of Congo (DRC), one of the largest countries in the region, adopted artesunate and amodiaquine (ASAQ) as first line antimalarial treatment in 2005. We conducted a randomised open-label non-inferiority trial, enrolling children aged 6-59 months with uncomplicated *P. falciparum* malaria in Pweto district, Katanga province. Patients were randomly allocated into one of the two regimens, fixed-dose formulation ASAQ or artemether-lumefantrine (AL). We analyzed the risk of recurrent parasitemia by day 42 adjusted by PCR genotyping, expressed as estimates of failure from survival analysis and as simple proportions (per protocol). Of 1993 children who were referred to the study clinic between April 2008 and March 2009, we enrolled 301 children: 156 with ASAQ and 145 with AL. The proportion of patients with parasitemia were low in both groups at D2 and D3: 6.0% (9/150) in the ASAQ arm and 4.9% (7/143) in the AL arm; and 0.6% (1/150) and 0.7% (1/143) respectively. After PCR correction, cure rates were 98.3% (95%CI, 94.1-99.9) in the ASAQ group and 99.1% (95%CI, 94.9-99.9) in the AL group (difference -0.7%, one sided 95%CI -3.1). Kaplan-Meier PCR-adjusted cure rates were similar: ASAQ, 98.4% (95%CI, 93.8-99.6) vs AL, 99.2% (95%CI, 94.3-99.9). Both treatment regimens were well tolerated. The results show that ASAQ was not inferior to AL and that both ACTs were highly effective as first-line malaria treatment and increased substantially the cost of the study. The recommended therapeutic efficacy surveys throughout the territory at repeated intervals are difficult to achieve considering the logistical challenges and the limited technical capacity in a country like DRC.

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**MONITORING THE EFFICACY AND SAFETY OF ACTS TO TREAT UNCOMPLICATED MALARIA IN BOBO-DIOULASSO, BURKINA FASO**

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Malaria in Burkina remains the major public health compromising therefore the development of the country. Since 2005, the national malaria control program advocated artéméther-lumefantrine (AL) and artesunate-amodiaquine (AS-AQ) respectively as first and second lines for the treatment of uncomplicated *falciparum* malaria. Monitoring efficiencies of these artemisinin based combination therapies play a major role in early detection and containment of resistance. We compared efficacies of AL and ASAQ for the treatment of uncomplicated *falciparum* malaria in two randomized trials with patients aged over 6 months. Outcome of treatment were defined according to standard WHO classification, ETF, LCF, LPF and ACPR. Genotyping to distinguish recrudescence from new infections is ongoing. Overall, 618 patients included in both studies completed their follow-up. We did not noted any ETF and at day 28, risk of recurrent infection were 9/66 (13.6%) in AL group compared to 4/62 (6.5%) in ASAQ group in 2009 and 46/211 (21.8%) compared to 20/215 (9.3%) in 2010. Most of treatment failures were new infections and PCR corrected ACPR were similar for both drugs in the two studies. No serious adverse event related to the studies drugs was recorded. Known polymorphisms-mediating resistance in pfcrt and pfmdr1 were not associated with treatment failure. All study drugs have shown excellent efficacy and safety in treating uncomplicated *falciparum* malaria in Burkina but the concern might be the reported resistance-mediating polymorphisms selection by the partner drugs following treatment.

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**ESTIMATING SELECTION ON *PLASMODIUM FALCIPARUM* DRUG RESISTANCE ALLELES IN AN ENDEMIC POPULATION OVER A 25-YEAR PERIOD**

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Using archived blood samples, we surveyed the changes in drug resistance alleles in The Gambia over a 25 year period from the time when resistance was unknown locally (in 1984) through periods of gradual failure of chloroquine therapy and increasing use of sulphadoxine-pyrimethamine until eventual introduction of artemisinin combination therapy (in 2008). At the first survey there were no drug resistance alleles detected at two of the loci (crt and dhps) and very few isolates contained resistance alleles at the other two loci (mdr1 and dhfr). Proportions of isolates with resistance alleles increased progressively over subsequent surveys, reaching peaks for the chloroquine resistance alleles crt 76T (76%) and mdr1 86N (78%) in the year 2000, and for antifolate resistance dhfr alleles (94%, mostly as a triple combination of 51I, 59C and 108N) and dhps 437G (86%) in 2007 and 2008 respectively (the dhps resistance allele 540E was not present in any of 623 isolates genotyped over the whole period). To estimate changes in allele frequencies over time, we counted one allele at each locus per isolate, randomly sampling when there were mixed genotypes, and estimated 95% confidence intervals based on sample sizes in each year. Changes in allele frequencies occurred at different times and rates over the period of survey, and the data fit closely a very simple model for each locus with assumed fitness costs and a change in selection.
coefficients reflecting historical change in therapeutic use. We explore the fit between these historical selection data and signatures of selection at these loci that can be derived from genome wide polymorphism data in a population sample taken at the end of the period.

ALIGNMENT AND GENE SET ENRICHMENT ANALYSIS OF TIME-COURSE PROFILES OF RECOMBINANT PfMDR1 AND PfCRT-MODIFIED PLASMODIUM FALCIPARUM PARASITE LINES

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Transcriptional profiling studies of the intraerythrocytic developmental cycle (IDC) of Plasmodium falciparum have revealed a unique transcriptome characterized by a continuous cascade of expression. Here we present a quantitative time-course analysis of the gene expression levels of 6 strains that differ in 2 key antimalarial resistance determinants, pfmdr1 and pfcr. Continuous expression profiles were imputed from the 8 time points sampled for each strain and then aligned through dynamic time warping. Transcriptional differences were elaborated at both the gene and gene set level using a novel algorithm that measures gene set enrichment at many discrete time points along the imputed and aligned expression profiles. Significantly up or down-regulated gene sets were identified in each comparison along with the time period of maximal enrichment. We present software to visualize the complete aligned expression profiles of each strain in 2 or 3 dimensions, facilitating comparison of individual time points as well as the full time-series. Comparison of our alignment methods with conventional techniques underscores the vital role that temporal alignment plays in discriminating genuine biological signal from the transcriptional noise created by gene expression cascades peaking at different time points and durations. Together, our data and software tools provide a window into the rich transcriptional complexity of P. falciparum parasites by allowing the alignment and comparison of strains that differ in fitness and therefore progress through the IDC at varying rates.

THE INTERACTION BETWEEN MALARIA PARASITES AND BLOOD GROUPS IN PORT HARCOURT, NIGERIA

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The impact of malaria on the public health of resource-limited economies of the world is still a major problem particularly in Africa where 89% of all malarial deaths occur. The pathogenesis of Plasmodium infection entails merozoite invasion of erythrocyte, which implies an implicit interaction between the red cell membrane proteins and the invading plasmodium antigens. Blood group antigens serve as genetic markers of several clinical conditions including malaria; the clearest example being the well elucidated inter-relation between Plasmodium vivax and the Duffy antigen. Thus, the products of research on blood groups and malaria may have a potential impact on the development of new anti-malarial chemotherapy, vaccines and reduction of the global burden of malaria. This study was designed to investigate the link between blood groups and different malaria parasites in Port Harcourt, Nigeria which is the centre of the oil and gas industry in West Africa. Furthermore, we will investigate the incidence of Plasmodium ovale and the specificity of the parasite strain in relation to various blood groups in this environment. Thick blood smears and filter paper blood spots were made from finger-prick for microscopy and molecular genotyping of parasite strains. Two hundred and forty six participants: 142 males (57.72%) and 104 females (42.28%) aged 16-60 years attending the Braithwaite Memorial Hospital and blood donors presenting at the University of Port Harcourt Teaching Hospital Blood Bank were enrolled into the study. Preliminary results showed that 207 (84.1%) were positive for Plasmodium falciparum while 39 (15.9%) were negative by microscopy. However prevalence of other species is expected from the PCR genotyping. Results of the blood group screening showed that blood group O Rh positive was the highest with 163 (66.2%) followed by blood group A Rh positive 43 (17.5%), B Rh positive 26 (10.6%), O Rh negative 7 (2.85%), AB Rh positive 5 (2.03%), B Rh negative 1 (0.41%) and A Rh negative 1 (0.41%).

BASOPHIL REACTIVITY IS ASSOCIATED WITH MALARIA SEVERITY AND PfTCTP

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Understanding of malaria immune-pathogenesis will lead to the identification of new therapeutic strategies aimed at improving recovery. Recent findings have suggested common mechanisms in malaria pathogenesis and allergy. Elevation of IgE levels has been associated with malaria infection, but their role remains unclear. Similarly, a parasite-derived histamine releasing factor (PfTCTP) was found at high level in serum from patients but in vivo effects are unknown. To address these questions, we conducted a clinical study in Dakar (Senegal). Plasmodium falciparum infected patients with mild (MM, n=19) or severe (SM, n=9) symptoms were enrolled and compared with healthy controls (HC, n=38). We performed basophil activation tests on whole blood samples based on CD203c expression to analyse allergic response. Basophils from MM patients showed significant lower baseline levels of CD203c expression, compared to SM and HC. Basophils from SM patients were characterized by a higher reactivity to A23187, haemozoin and anti-IgE stimulation. Ex vivo priming of basophils with recombinant human or PfTCTP before stimulation with anti-IgE induced either an enhancement or an unexpected decrease in activation (mostly in MM and HC patients). The decrease in basophil activation, previously described as an “overstimulation”, suggests a better ability of HC and MM patients to control allergic response following excessive stimulation. IgE levels were also higher in malaria patients than in healthy ones, but were not related to basophil responses. Indeed the reactivity of basophils from malaria patients was positively related to the presence of circulating PfTCTP or for SM, to the lack of anti-PfTCTP IgG. Altogether these data revealed a high reactivity of basophils during SM which could explain the high level of histamine reported during SM, likely contributing to blood-brain-barrier impairment. These findings support an involvement of allergic immune responses in malaria pathogenesis which can be exacerbated by the proinflammatory environment and PfTCTP.

IPT/C: PREVALENCE OF ANTIBODY AMA-1 AND MSP-1(19) IN THREE AREA IN SENEGAL

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Malaria remains a major disease in many African countries. Nowadays, many strategies such as IPTi/SP are used for prevention in children. But SP-resistant parasites can compromise this strategy. To evaluate the impact of IPTi/SP on antigenic variation in rural areas of three districts, all children aged 5-10 years, in Senegal. In 2009, to assess the role of
serological markers in evaluating malaria transmission, filter papers were collected from children under 10 years. Filter blood spot papers were collected from 5833 people from Mbour, Bambe and Fatick to assess the prevalence of antibodies to two Plasmodium falciparum antigens MSP-1(19) and AMA-1. Seropositivity to P. falciparum MSP-1(19) was 15.5 % and 26.7% to AMA-1. MSP-1(19) is lower than AMA-1. Fatick presents most of positive children who answer to antibody. Also in Fatick the young children have least antibody. Seroprevalence can provide key information on malaria transmission for control programmes, when parasite rates are low.

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COMPARATIVE PROTEOMIC ASSESSMENT OF PLASMODIUM CHABAUDI ADAMI AS-INFECTED AND NAÏVE MOUSE SERUM TO IDENTIFY CANDIDATE CFF PROTEINS

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The identity of serum crisis factor (CFF) has remained elusive for decades after its initial characterization as a factor in human immune sera able to inhibit growth and cause the intraerythrocytic degradation of the malaria parasite, Plasmodium falciparum, in culture. CFF is named for the association of its coincident presence with the immunological crisis leading to resolution of infection and is inducible through artificial immune stimulation in rabbit and rodent models by treatments such as BCG or malaria infection. CFF has been described in the serum of some individuals with apparent resistance to malaria symptoms and may provide a novel mechanism of natural immunity. Identification of CFF would enhance our understanding of how immune system components interact with a P. falciparum infection to produce acquired immunity, which is a critical component of the current investigation into a malaria vaccine. In this study, we used the C57Bl/6 mouse model inoculated with P. chabaudi adami AS to induce serum CFF, as documented by inhibition of P. falciparum growth in culture and the appearance of classic CFF responses in microscopic findings. We isolated the low abundance protein fraction of these CFF mouse sera using IgY depletion. Proteinomic analysis using MALDI and LC-QToF was conducted on depleted serum from the C57Bl/6 P. chabaudi adami AS model and non-inhibitory serum from naïve mice. Protein differences were quantified to discover proteins that were present in the CFF serum and absent from naïve serum. This analysis highlighted 68 proteins as either up-regulated or unique to CFF serum, were present in the CFF serum and absent from naïve serum. This analysis naïve mice. Protein differences were quantified to discover proteins that C57BL/6 P. chabaudi adami AS model and non-inhibitory serum from naïve mice. Protein differences were quantified to discover proteins that CFF mouse sera using IgY depletion. Proteomic analysis using MALDI and LC-QToF was conducted on depleted serum from the C57Bl/6 P. chabaudi adami AS model and non-inhibitory serum from naïve mice. Protein differences were quantified to discover proteins that were present in the CFF serum and absent from naïve serum. This analysis highlighted 68 proteins as either up-regulated or unique to CFF serum, were present in the CFF serum and absent from naïve serum. This analysis

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PEDIATRIC MALARIAL ANEMIA SEVERITY IS DEFINED BY ELEVATED LEVELS OF CIRCULATING MEMORY CD4 T CELLS PRODUCING IL-17

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In Plasmodium falciparum holoendemic transmission regions of western Kenya, severe pediatric malaria manifests as severe malarial anemia (SMA). We hypothesized that children presenting with SMA would have chronic immune responses characterized by effecter/central memory CD4+ T cells producing interferon (IFN)-γ and/or interleukin (IL)-17 that suppressed their erythropoietic responses. We therefore characterized the CD4+ T cell populations and their intracellular IFN-γ and IL-17 production in healthy controls [HC; hemoglobin (Hb)<11.0g/dL, without parasitemia, n=13] and febrile children with differing levels of malarial anemia severities and any density parasitemia: uncomplicated malaria (UM, Hb=11.0g/dL, n=140; mild malarial anemia (MMA, Hb=8.0s-10.9g/dL, n=23); and SMA (Hb<6.0g/dL, n=23). Across group comparisons revealed that children with SMA had elevated effector memory (TEM) cells [median (IQR) 92.60% (75.50) relative to the HC [75.00% (19.10)], UM [62.80% (15.50)], and MMA [66.70% (25.00), P<0.001] groups, TEM cells were present in the CFF serum and absent from naïve serum. This analysis naïve mice. Protein differences were quantified to discover proteins that were present in the CFF serum and absent from naïve serum. This analysis highlighted 68 proteins as either up-regulated or unique to CFF serum, were present in the CFF serum and absent from naïve serum. This analysis

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IDENTIFICATION OF HOST TRANSCRIPTIONAL PROFILES ASSOCIATED WITH ASYMPTOMATIC MALARIA AFTER A BOUT OF SEVERE MALARIA

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Clinical signs of Plasmodium falciparum can range from cerebral malaria to asymptomatic carriage. Prior exposure and host genetics will alter clinical presentation; however, the mechanisms associated with clinical presentation are not fully characterized. To explore the role of the host response across clinical phenotypes, we studied human whole genome transcription expression profiles from children with cerebral malaria admitted to the Blantyre Malaria Project during a single malaria season. Survivors are invited to return for a one-month follow-up visit, and we analyzed samples from survivors found to have asymptomatic malaria infections at that time. Whole blood (2-3 mL) was collected, stabilized in Tri-Reagent, and frozen at -80°C at the time of admission and at follow up (day 30). RNA was isolated from sixty severe disease samples and five follow-up matched samples. RNA was hybridized to Affymetrix GeneChip Human 1.0 ST Arrays. For the paired analysis of the severe and asymptomatic samples (n=5), we identified significantly differential gene sets using GSEA (GenePattern, Cambridge, MA) software. The severe disease presentation in the matched samples was significantly associated with olfactory sensory transcripts (GO:olfactory sensory receptor activity). The olfactory bulb is unique to the central nervous system in that it has an external component. We speculate that our peripheral blood analysis may be detecting this peripheral component of the brain, reflecting the central nervous system abnormalities involved in cerebral malaria. GO sets significantly upregulated in samples derived from the asymptomatic presentation reflect immune system upregulation (GO:regulation of the immune system processes; GO:regulation of leukocyte differentiation). This is the first report that captures the peripheral blood transcriptomes during a bout of severe malaria and during a subsequent asymptomatic infection
and may provide insight into host response associated with clinical presentation to inform pathogenesis/immunity models and potential targets of intervention.

1196
CD11C EXPRESSION DEFINES MULTIPOTENT EFFECTOR MEMORY CD8 T CELLS INDUCED BY GENETICALLY-ATTENUATED MALARIA VACCINES
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Vaccination with live, genetically-attenuated Plasmodium yoelii parasites (PyGAPs) can induce long-lasting sterile protection against liver stage malaria in mice with just one dose. The underlying mechanisms mediating this protective immune response are not fully understood, but further characterization will be vital for guiding future vaccine design. Previous work from our lab demonstrates that protective immunity following PyGAP immunization is completely dependent on CD8+ T cells, partially dependent on IFN-γ and perforin, and likely mediated by direct cytotoxic killing of parasite-infected hepatocytes. In addition, protective efficacy correlates with expansion of effector memory CD8 T cells in the liver. We went on to further characterize vaccine-induced changes in the T cell phenotype and found significant up-regulation of CD11c on CD3+CD8+ T cells in the liver, spleen and peripheral blood. As much as 50% of CD8 T cells co-expressed CD11c in the liver, which is the site of infection, and expansion of the CD11c+ CD8 T cell population correlated with protective efficacy following various vaccine regimens. CD11c expression was specifically induced on T cells from immunized mice but not from control mice following co-culture with malaria-infected hepatocytes. Further analysis demonstrated that these CD11c+ T cells are predominantly CD11a− CD44+ CD62L−, indicating that they are antigen-experienced, effector memory cells. Following in vitro re-stimulation with malaria-infected hepatocytes, CD11c+ CD8 T cells expressed multiple inflammatory cytokines and cytotoxicity markers, including IFN-γ, TNF-α, IL-2, perforin and CD107a. CD11c- CD8 T cells, on the other hand, expressed negligible amounts of inflammatory cytokines and cytotoxicity markers, indicating that CD11c expression accurately defines multifunctional effector CD8 T cells. Surprisingly, we found that CD11c+ CD8 T cells also express other antigen-presenting cell (APC) markers, including MHC class II, CD80 and CD86, suggesting that these cells may have an unusual APC-like phenotype. Taken together, our data demonstrate that CD11c+ CD8 T cells are multipotent effector memory cells that are likely to mediate the protective immune response against liver stage malaria infection following PyGAP vaccination.

1197
DECREASED SYSTEMIC PROSTAGLANDIN (PG)-E2, AND CYCLOOXYGENASE (COX)-2 GENE EXPRESSION IN CHILDREN WITH SEVERE MALARIA ANEMIA AND CO-INFECTION WITH HIV-1 OR BACTEREMIA
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In malaria endemic regions of western Kenya, Plasmodium falciparum malaria manifests clinically as severe malaria anemia (SMA; hemoglobin (Hb)<6 g/dL, any density parasitemia). Although we have previously shown that prostaglandin (PG)-E2, cyclooxygenase (COX)-2 transcripts and protein levels are reduced in children with severe and cerebral malaria, the impact of HIV-1 and bacteremia co-infections, and in vivo malaria pigment containing monocytes (PCM) on systemic PGE2, production and COX-2 mRNA expression in children with SMA has not been investigated. As such, we investigated plasma and urine PGE2, (measured as bicyclo-PGE2) and COX-2 mRNA expression in children with clinical malaria (n=74) and those co-infected with either HIV-1 (Pf+/HIV-1+; n=8) or bacteremia (Pf+/bacteremia+; n=19). Plasma (P=0.001) and urinary (P=0.001) PGE2 levels were decreased in children with SMA relative to the non-SMA (Hb<6 g/dL, any density parasitemia) group. Additionally, PGE2 levels were lower in Pf+/HIV-1+ children in plasma (P<0.001) and urine (P=0.007), as well as Pf+/bacteremia+ children in plasma (P=0.001) and urine (P=0.173), relative to those with malaria infection alone. PGE2 increased with increasing hemoglobin levels in children with malaria (plasma; r=0.363, P=0.002 and urine; r=0.500, P=0.001), and in co-infected children (Pf+/HIV-1+; r=0.819, P=0.013 and Pf+/bacteremia+; r=0.595, P=0.007). Additional analyses demonstrated decreasing PGE2 levels with increasing PCM in plasma (P=0.031) and urine (P=0.070). COX-2 mRNA expression was decreased in children with SMA relative to the non-SMA group (P=0.011) and in Pf+/bacteremia+ (P=0.033) and Pf+/HIV-1+ children (P=0.118) relative to those with malaria alone. Taken together, results demonstrate that SMA is associated with decreased PGE2, and COX-2 gene expression, and is further augmented by co-infections (HIV-1 and bacteremia), driven in part, by naturally acquired malarial pigment by monocytes.

1198
LIVER-RESIDENT CD8+ T CELLS INDUCED BY RADIATION-ATTENUATED PLASMODIUM SPOROZOITES
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Memory CD8+ T cells induced by malaria sporozoites home to the liver and eliminate parasite-infected hepatocytes. While memory T cells residing in lymph nodes, spleen, lung and peripheral blood are polyfunctional, capable of mediating cytotoxicity and producing multiple cytokines, the liver-resident memory cells exhibit a unique mononuclear profile with normal cytotoxic activity but minimal cytokine production. This phenotype is specifically induced by parasites but not viruses expressing the same epitope. The liver-resident memory cells are not exhausted, anergic or senescent, albeit their proliferation after in vivo antigen re-exposure is markedly reduced. Importantly, these cells undergo vigorous homeostatic proliferation, display normal in vivo cytotoxic activity and inhibit parasite development in hepatocytes. Surprisingly, these cells are fully capable of producing IFN-γ transcripts but translation occurs only in response to TCR-independent stimuli. These results suggest that parasite-induced liver-resident memory CD8+ T-cells represent a distinct terminal effector lineage characterized by a mononuclear profile maintained in part through translational control of cytokine production.

1199
USING PROTEIN ARRAYS FOR ANTIBODY PROFILING AND DISEASE STRATIFICATION IN MALARIA INFECTION
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The prevalence of mixed-species malaria infections was underestimated until more sensitive detection methods, such as PCR-based diagnosis, were introduced for epidemiological studies in malaria endemic areas. In the era of malaria elimination, improved diagnostic tools are required to enable targeted treatment of infected individuals as well as effective mass screening for the detection of very low parasite densities to monitor
transmission reduction. Serological markers represent a promising tool for diagnostics and surveillance, especially for *Plasmodium vivax*, for which current rapid diagnostic tests are less effective. A comprehensive characterization of the antibody response to blood stage malaria for both *P. falciparum* and *P. vivax* is required for the discovery of novel markers of both single and mixed clinical infections, as well as asymptomatic low density infections. Using recent developments in malaria genomic sequencing, proteomics, bioinformatics, high throughput cloning and proteome microarray fabrication technologies, we have constructed a blood stage proteome antigen array with a total of 4,000 recombinant proteins, which are the expression products of approximately 2,000 *P. falciparum* and 2,000 *P. vivax* blood stage ORFs. After recombinant cloning proteins were expressed using an E. coli based cell free expression system and printed directly on the nitrocellulose coated microarray slides without purification. Using this protein chip, sera from both symptomatic and asymptomatic children with *P. falciparum* and/or *P. vivax* infections from Papua New Guinea were screened. This approach will provide new insights into the correlation between antibody profiles and disease states that will lead to the characterization of serological correlates of active and past infection. These proteins are potential biomarkers that can be used for the development of diagnostic tools for the detection and characterization of co-infections, or for sero-surveillance markers.

**1200**

**NO CORRELATION BETWEEN PARASITEMIA AND IGG ANTIBODY RESPONSE AGAINST PLASMODIUM FALCIPARUM GLUTAMATE-RICH PROTEIN (GLURP-R2) IN SERUM SAMPLES OF PATIENTS FROM IQUITOS, LORETO**

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The highly antigenic *Plasmodium falciparum* GLURP-R2 protein is expressed in all stages of the parasite life cycle in human. It is considered as an important vaccine candidate antigen because its interaction with human IgG may play an important role in the development of clinical immunity. The aim of this study was to evaluate the IgG antibody response induced by GLURP R2 antigen in serum samples of patients infected with *P. falciparum* by indirect ELISA. Serum samples from 47 patients, between 9 and 63 years-old, mostly adults, infected with *P. falciparum*, were collected mainly in San Juan and Atalaya districts (province of Maynas), department of Loreto. All samples were positive by PCR and microscopy. Most patients from Atalaya community were asymptomatic, who showed low levels of parasitemia (from 24 to 7477 parasites/µL), while other communities showed higher levels of parasitemia (from 2162 to 61185 parasites/µL). Eight samples of people without any history of malaria disease were used as negative controls. Serum from patients infected with *P. vivax* was used to confirm the specificity of the assay. The cutoff value was calculated using the mean Optical density (OD) plus three standard deviations of negative control group. 87.23% (41/47) and 12.77% (6/47) were seropositive and seronegative to GLURP R2, respectively. The statistical significance of the correlation between parasitemia and IgG response level in both seropositive and seronegative groups was calculated with 95% of confidence (p<0.05). There was a weak inverse correlation between IgG response versus Log (parasites/µL) (R²=0.303) for the seropositive group and a direct correlation for the seronegative group (R²=0.786). In addition, there was no correlation between the IgG response and parasites/µL neither with age or sex of the patient. In conclusion, the absence of significant correlations found shows that the immune response is influenced by other factors either intrinsic or extrinsic to the patient and that GLURP would not be a good vaccine candidate applicable to this region.

**1201**

**PLASMODIUM FALCIPARUM DRUG RESISTANCE MOLECULAR MARKERS UNDER INTERMITTENT PREVENTIVE THERAPY WITH DITHYDROARTEMISININ-PIPERAQUINE (DP) VS. AMODIAQUINE-SULFADOXINE/PYRIMETHAMINE (AQ-SP) IN BURKINA FASO**

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Single nucleotide polymorphisms (SNPs) in the *Plasmodium falciparum* pfcrt, pfmdr1, pfdhfr and pfldhps genes are associated with decreased response to amodiaquine and antifolate antimalarials and have been shown to be selected by use of these drugs. The degree of selection by intermittent preventive therapy (IPT) regimens is unknown. We assessed the baseline prevalence and selection of common SNPs by IPTc in children in Bobo-Dioulasso, Burkina Faso. We studied 1500 children (aged 3-59 months) randomized to receive monthly dithydroartemisinin-piperaquine (DP) or amodiaquine-sulfadoxine/pyrimethamine (AQ/SP) for 3 months during the malaria transmission season in 2009. From random samples of 120 children for each arm of the study and for 120 of 250 untreated controls we evaluated the prevalence of key resistance-mediated SNPs. We then assessed the prevalence of the same SNPs in samples collected in November, 1 month after the third dose of IPTc. Before therapy malaria prevalence was 52.2% (188/360) based on microscopy and 66.67% (240/360) measured by PCR. Prevalences of SNPs were 68.5% (178/260) for pfcrt 76T, 29.1% (75/258), 58.5% (151/258) and 7.70% (20/260) for Pfmdr1 86Y, 184F and 124Y, respectively; 58.1% (151/260), 54.8% (143/261), and 55.0% (143/260) for Pfdhfr S196N, S108N, respectively; and 35.1% (91/259) and 56.8% (147/259) for Pfdhps 436S and 437G. After three monthly IPTc, AQ-SP selected significantly for mutant sequence pfcrt 76T, pfdhfr S196N, S108N and triple mutant S196N, S108N and 108N. DP did not select for known polymorphisms associated with aminoquine and antifolate resistance. Our result indicated that IPTc with AQ-SP selected for polymorphisms linked to resistance to AQ and SP probably because of increasing use of these drugs. IPTc with DP do not select for known polymorphisms associated with drug resistance. DP may therefore be an excellent alternative for malaria prevention in children in Burkina. Nevertheless, further investigations are needed to confirm this absence of resistant parasite selection following IPTc with DP.

**1202**

**IDENTIFICATION OF A KUPFFER CELL RECEPTOR FOR PLASMODIUM SPOROZOITE RECOGNITION**

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After inoculation by the bite of an infected mosquito, the *Plasmodium* sporozoite enters the blood stream and infects the liver with unique specificity. To establish a productive hepatocyte infection sporozoites must find and traverse a Kupffer cell, a macrophage-like cell that lines the liver sinusoids. Using a phage display library we identified the P39 peptide bound specifically to a ~110 kDa Kupffer cell membrane protein. We determined that the P39 peptide binds specifically to ~110 kDa Kupffer cell membrane protein and hypothesize that this protein acts as a sporozoite receptor for Kupffer cell traversal.

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CONTRASTING PATTERNS OF SELECTION ON THE ORTHOLOGOUS GENES ENCODING MEROZOITE SURFACE PROTEINS 4 (MSP-4) AND 5 (MSP-5) IN PLASMODIUM SPP.

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Merozoites are the invasive form of the malarial blood-stage life-cycle, exposing the merozoite surface proteins (MSPs) on their surface that are involved in initial attachment to the erythrocytes. Given the role of these proteins during the invasion of the red blood cell (RBC), several of them have been considered potential vaccine candidates. Among the MSPs, Merozoite surface protein 4 (MSP-4) and 5 (MSP-5) have received attention since these proteins share crucial structural features. They are glycosylphosphatidylinositol (GPI)-anchored integral membrane proteins with one epidermal growth factor-like domain (EGF) at the C-terminal. In addition, the genes encoding MSP-4 and MSP-5 are closely linked on the genome downstream from the gene encoding the highly conserved enzyme adenylosuccinate lyase (ASL). A single protein (MSP4/5) considered similar to both proteins has been identified in the three rodent malaria species; such a gene has lead to the hypothesis that MSP-4 and MSP-5 originated as a result of an early duplication event. In this study, we investigated the genetic diversity of orthologous genes encoding the MSP-4 and MSP-5 among Plasmodium species found in non-human primates that are closely related to P. vivax. We also evaluate the hypothesis that these genes are the result of an early duplication event during the evolution of Plasmodium in mammals. Overall, we found contrasting patterns of selection acting in genes encoding MSP-4 and MSP-5 in P. vivax and related species; MSP-5 orthologs are twice as polymorphic as MSP-4. In addition, we found that the polymorphism in MSP-4 in all Plasmodium species included in this study appears to be neutral. In contrast, we found evidence suggesting that MSP-5 in P. vivax, P. cynomolgi and P. inui is under positive selection. Our results reveal that exon I exhibits significant more non-synonymous than synonymous substitutions, confirming previous reports in P. vivax. This finding suggests that MSP-5 may be under selective pressure by the immune system across different species of primates including humans.

EXTERNAL QUALITY ASSURANCE PROGRAM FOR PLASMODIUM FALCIPARUM RECRUDESCENCE-REINFECTION GENOTYPING IN ANTIMALARIAL DRUG EFFICACY STUDIES

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The authors present this on behalf of the Molecular Surveillance Network for Malaria Drug Resistance in the Greater Mekong Subregion.
Analysis of Field Isolates from a Chronic Plasmodium Falciparum Infection Suggests That Variant Surface Antigens Are Not Exclusively Composed of PFEMP1

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Antigenic variation of variant surface antigens (VSA) enables Plasmodium falciparum to establish chronic infections. Plasmodium falciparum erythrocyte membrane protein 1 (PFEMP1) is suggested to be the major cause of antigenic variation. PFEMP1 is encoded by the multicopy var gene family. We have shown that var gene transcription in the 3D7 genome strain and in field isolates is biased towards central UpsC var genes (Enderes et al. submitted). This raises the question how P. falciparum escapes the immune response if it constantly expresses an individual var locus. Here we employ parasites and sera from an asymptptomatically infected individual to investigate the determinants of antigenic variation. We used shotgun cloning to characterize the var gene repertoire at different time points of the infection. Fluorescence activated cell sorting (FACS) was employed to characterize the humoral immune response. To determine individual targets of the antibody response we generated PFEMP1 knock-down parasites in field isolates as well as in NF54 laboratory clones. In these parasites drug pressure removes PFEMP1 from the erythrocyte surface. CD36 receptor binding was used to select for PFEMP1 expression in all parasite lines. The var gene repertoire was identical at all time points of the infection, underscoring the parasites ability to evade the human immune response. FACS with sera of the infected individual displayed a strong signal in culture adapted field parasites but not in NF54. However, selection for CD36 binding induced a strong FACS signal in NF54 parasites suggesting crossreactivity with VSA. Application of drug pressure to transgenic NF54 parasites removed this signal, indicating that these antibodies are directed against PFEMP1. Surprisingly, drug pressure had no effect on the FACS signal of transgenic parasites. This suggests that a large part of the epitopes on these field isolates are not PFEMP1. Taken together our data suggest that antigenic variation is not exclusively mediated by PFEMP1. Transgenic field isolates may provide new insights into the mechanisms mediating immunity to P. falciparum malaria.

Population Genetic Inferences of Plasmodium Falciparum Based on Fully Sequenced Genomes from Senegal

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Malaria is a deadly disease that causes nearly one million deaths each year. Understanding the demographic history of the malaria parasite Plasmodium falciparum and the genetic basis of its adaptations to antimalarial treatments and the human immune system is important for developing methods to control and eradicate malaria. To study the demographic history and identify genes under selection more efficiently, we sequenced the complete genomes of 25 cultured P. falciparum isolates from three cities in Senegal. Based on genetic diversity of the genome sequences, we estimate the long-term effective population size to be approximately 100,000 and show that there is no significant population structure within Senegal. We also estimate a major population expansion of the parasite population approximately 550,000-770,000 years ago. By using the results on demographic history as a null model, the sequences also reveal candidate genes under selection, including pfcr and dfr. Moreover, the rates of decay of linkage disequilibrium are fast, indicating the potential of fine-scale genetic mapping in P. falciparum.
analysis, including long-range haplotype tests for positive selection, to
determine whether these regions represent additional sweeps or areas of
reduced recombination.

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INVASION OF PLASMODIUM FALCIPARUM FIELD ISOLATES
FROM SOUTH AMERICA: PHENOTYPIC AND GENOTYPIC
ANALYSES

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Invasion of RBCs by Plasmodium falciparum involves multiple pathways
including those utilizing ligands of the Erythrocyte-Binding Ligand (EBL)
and the Reticulocyte-Binding protein homolog (PFRh) families. The invasion
of 20 South American (SA) field isolates from Colombia (Antioquia),
Peru (Iquitos) and Brazil (Pará) was studied. Seven different invasion
profiles were found, one of which is independent of neuraminidase (N),
trypsin (T) and chymotrypsin (C) sensitive receptors (NrTcC), and which
was not previously reported. This pathway was used predominantly by
Colombian and Peruvian field isolates with varying levels of resistance to
the three enzyme treatments (58-93%). Regrettably, majority of other
invasion studies did not examine the chymotrypsin treated RBCs for
their invasion profile classification. However, even when only the use of
the NrTr invasion pathway was compared between the SA isolates and
those studied previously, it appeared that 46% of the SA isolates use
this pathway in contrast to <5% by African and Brazilian (Mato Grosso)
isolates. The use of chymotrypsin treated RBCs allowed us to evaluate
the involvement of GPB, and the unknown receptors of EBA-181, PFRh2b
and PFRh4 in the alternative invasion pathways of the SA isolates, and
which appeared to be more predominant in the Brazilian isolates (5/7). The
specific dependence on GPB for invasion was further estimated by using
GPB-negative RBCs and the differential use of this receptor vs. the other
chymotrypsin sensitive receptors will be presented. Two distinct dominant
clusters of invasion profiles were found in SA field isolates: NrTsCs in
Brazil, and NrTcC in Colombia and Peru, both of which are different of
those present in Africa, and in part more similar to the Indian field isolates.
When the polymorphic variants of the PFRh and EBA-181 and EBL-1
ligands were compared to lab strains and the Mato Grosso field isolates,
we found some novel variants in the Peruvian and Colombian field
isolates. The association between ligand polymorphisms and the differing
invasion pathways used by the SA parasites will be discussed.

1211

USING CF11 CELLULOSE COLUMNS TO QUICKLY,
INEXPENSIVELY AND EFFECTIVELY REMOVE HUMAN DNA
FROM PLASMODIUM FALCIPARUM-INFECTED WHOLE
BLOOD SAMPLIES

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Next-generation Illumina® sequencing of Plasmodium genomes requires
depletion of human DNA from parasitized whole blood samples prior
to extraction, storage and shipping of DNA to sequencing facilities.
The most effective method currently in use is a two-step procedure
deplete leukocytes: centrifugation using density gradient media
followed by gravity filtration through expensive, commercially-available
columns. This method is not easily implemented in studies collecting
hundreds of samples, processing samples for multiple laboratory analyses
simultaneously, or lacking capacity for refrigerated centrifugation.
Inexpensive syringes hand-packed with CF11 cellulose powder were
recently shown to improve ex vivo cultivation of Plasmodium vivax
obtained from parasitized whole blood samples, as reported previously.
We have adapted this procedure to isolate P. falciparum DNA from in vitro
cultured parasites and parasitized whole blood samples obtained ex vivo
from Cambodian patients with malaria. Using this method to process
blood samples of at least 2 mL and containing at least 10,000 parasites
per microliter, we reliably produced 500 nanograms of parasite DNA
with less than 30% human DNA contamination. This sample profile is
comparable to that obtained by the two-step method and falls well within
the current quality control requirements for Illumina® sequencing. In
addition, we have validated a centrifuge-free version of the CF11 filtration
method to isolate P. falciparum DNA at remote and minimally-equipped
sites in malaria-endemic areas.

1212

GENETIC DIVERSITY IN PLASMODIUM FALCIPARUM MSP
GENE FOR 7 DAYS POST-TREATMENT CHARACTERIZE
TREATMENT FAILURES IN AN ARTESUNATE MONO-THERAPY
TRIAL IN WESTERN CAMBODIA

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Diversity in the Plasmodium falciparum genes encoding merozoite
surface proteins (msp) msp1 and msp2, and glurp, has implications for
the epidemiology of malaria and the efficacy of malaria drugs. The WHO
standard for determining whether a treatment failure is a recrudescence
or a new infection uses matched samples from baseline (D0) and day
of failure (D). However, in a region of emerging drug resistance, the
proportion of parasite at D, susceptible to drug may be large and mask a
minute proportion of parasites that are drug resistant. We hypothesized that a small population of resistant parasites may escape drug therapy undetected and reemerge later at the time of treatment failure. In this scenario, a specimen from Day 3 of treatment would better represent parasites that are more resistant to drug and that may actually lead to treatment failure. In a randomized study conducted in an area of emerging artemisinin resistance in western Cambodia during 2008-2009 the efficacy of 7-day courses of artesunate monotherapy for the treatment of uncomplicated falciparum malaria were assessed. Samples for nested PCR were collected pre-treatment, on days 2, 3, 4, 5 and 6 of treatment, and on the day of failure (D). Patterns of allelic diversity of msp and glurp were used to distinguish between recrudescence and reinfection by nested PCR. 143 patients were enrolled of who 10 were classified as late treatment failures, 2 as reinfection and 8 recrudescent. A high proportion of isolates from recrudescent subjects showed multiple msp allelic types on D0 and Df, consistent with a heterogeneous falciparum infection. For some subjects, in comparison with D0, some alleles disappeared by day 3, and re-appeared on Df. In conclusion, for assessing re-infection of uncomplicated malaria, a specimen from Day 3 of treatment would better represent a small population of resistant parasites may escape drug therapy undetected and reemerge later at the time of treatment failure. In this scenario, a specimen from Day 3 of treatment would better represent parasites that are more resistant to drug and that may actually lead to treatment failure. In a randomized study conducted in an area of emerging artemisinin resistance in western Cambodia during 2008-2009 the efficacy of 7-day courses of artesunate monotherapy for the treatment of uncomplicated falciparum malaria were assessed. Samples for nested PCR were collected pre-treatment, on days 2, 3, 4, 5 and 6 of treatment, and on the day of failure (D). Patterns of allelic diversity of msp and glurp were used to distinguish between recrudescence and reinfection by nested PCR. 143 patients were enrolled of who 10 were classified as late treatment failures, 2 as reinfection and 8 recrudescent. A high proportion of isolates from recrudescent subjects showed multiple msp allelic types on D0 and Df, consistent with a heterogeneous falciparum infection. For some subjects, in comparison with D0, some alleles disappeared by day 3, and re-appeared on Df. In conclusion, for assessing re-infection and recrudescence in malaria treatment trials, the inclusion of msp allelic analysis on post-treatment days 2 through 7, especially day 3, may be a useful addition to the current WHO standard of D0 and Df for characterizing allelic distribution.

1213

APPLICATION OF NEXT GENERATION SEQUENCING TO SEARCH FOR ALLELE-SPECIFIC IMMUNITY IN AN RTS,5 CLINICAL TRIAL IN MOZAMBIQUE?

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Most candidate malaria vaccines, including the RTS,5 vaccine that is now undergoing Phase III trials in Africa, are subunit protein vaccines based on highly polymorphic loci. The goal of this study is to understand the impact of vaccination on the diversity of the parasite population in individual patients. The underlying hypothesis is that successful vaccination is allele-specific and we have tested this hypothesis by comparing parasite populations in RTS,5 vaccinated versus control vaccinated individuals. Specifically, we have analyzed the association between parasite diversity at the CSP locus, the antigen target of the RTS,5 vaccine, and vaccination in patients who contracted infection during a Phase IIb RTS,5 trial conducted in Mozambique. One of the key technical developments necessary to conduct this work is the ability to both detect and quantify the parasite populations within a single patient. To achieve this goal, we developed a new genotyping approach that utilizes next generation sequencing to recover CSP haplotypes in both mixed and single clone infections. Haplotypes capture complex interactions between polymorphisms and are directly associated with protein production and parasite fitness. The sequenced reads were filtered to remove error-prone and misaligned reads and then clustered by similarity into haplotypes. Using model-based filters, we identified and removed SNP errors and chimeras that arise during sample preparation and sequencing and further improved the accuracy of haplotype identification. This approach successfully recovers rare haplotypes (at a frequency of 1%) and yields a sensitive measurement of multiplicity of infection (MOI). The approach has been validated in in vitro mixtures of parasites. We assessed whether the parasite populations were different between RTS,5-vaccinated and comparator-vaccinated subjects with respect to specific haplotypes and MOI. Additionally, we re-visited the Enosse et al (2006) analysis to assess whether the increased efficacy of the RTS,5 vaccine against severe disease could be attributed to a decrease in MOI. This analysis strategy should prove useful for evaluating allele-specific efficacy in the context of other malaria vaccine trials.

1214

TRANSMISSION BLOCKING ACTIVITY OF ANTIBODIES RAISED AGAINST A PFSP25- BASED VACCINE DERIVED FROM NF54 SEQUENCE AGAINST FIELD ISOLATES FROM THAILAND

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Eradication of malaria is possible by the interruption of local mosquito borne malaria transmission and is the end goal in the fight against the disease. Transmission-blocking vaccines (TBVs) that target the sexual stage react with the ookinete surface proteins of malaria parasites within the mosquito midgut, which will contribute to elimination of the disease by blocking the parasite transmission. Pfs25 is a lead TBV candidate, and a Pfs25-based vaccine, Pfs25/ISA51 has been tested in a Phase 1 trial. The vaccine was produced using the Pfs25 sequence from NF54 isolate. Since only a limited sequence polymorphism was reported for this gene, we hypothesized that anti-Pfs25 antibodies induced by this vaccine will have transmission blocking activity against most, if not all, field isolates. To test this hypothesis, we evaluated transmission blocking activities of anti-Pfs25 plasma from the Pfs25/ISA51 trial against parasites in blood of Plasmodium falciparum infected patients in Thailand. Normal human Plasma was used as controls. The blood was drawn from each patient and was first tested for transmission blocking activity by membrane feeding assay in triplicates. In parallel, blood samples from these patients were spotted on filter papers for sequencing of Pfs25 genes and for genotyping analysis to determine the independent origin of the parasites. Despite the different genetic background, the Pfs25 sequences from these parasites are identical. The transmission blocking activities of the plasma against these parasites in different blood samples are comparable. Percent reduction in oocyst count in membrane feed assay, when immunized plasma compared with normal plasma is highly significant (P=0.0001).

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CLINICAL TRIAL OF THE SANARIA® PFSPZ VACCINE VIA THE INTRAVENOUS ROUTE - RATIONALE, PLANS AND PROGRESS

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Immunization by the bites of mosquitoes infected with radiation-attenuated Plasmodium falciparum sporozoites remains the most effective method for inducing sustained, high-level protective immunity in humans not treated with antimalarials. To advance this concept, the Plasmodium falciparum Sporozoite (PFSZ) Vaccine, comprising metabolically-active, non-replicating, purified, aseptic, cryopreserved parasites, has been developed. In the first human trial of the PFSZ Vaccine, immunization of healthy malaria-naive volunteers by the subcutaneous (SC) and intradermal (ID) routes was safe and well-tolerated. However, both immunogenicity and protective efficacy were suboptimal. Recent experiments in mice, rabbits, and especially non-human primates (NHPs) demonstrate that the PFSZ Vaccine is highly potent and that immunogenicity and protective efficacy are far superior when administered intravenously (IV) as compared to SC or ID. In NHP, high levels of SPZ specific CD8+IFN-g producing cells

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were detected in the livers several months after a series of IV but not SC immunizations. In vitro data demonstrate that irradiated, aseptic, purified, cryopreserved PSSPZ can invade NHP hepatocytes, providing a potential explanation for such potent responses. Furthermore, administration of labeled SPZ in mice confirm substantially greater distribution of the vaccine to the liver after IV than after SC administration. Together, these animal studies provided the rationale for assessing IV administration of the PSSPZ Vaccine in a Phase 1 clinical trial with experimental challenge. This dose escalation trial is designed to maximize volunteer safety and to provide 1) a clinical proof of principle, 2) a foundation for a clinical development plan leading to licensure of IV-administered vaccine for targeted market segments and 3) a benchmark for development of a non-IV parenteral mode of administration.

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VACCINE CANDIDATE IDENTIFICATION FOR PEDIATRIC FALCIPARUM MALARIA

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Plasmodium falciparum remains a leading cause of morbidity and mortality in developing countries and vaccines for this parasite are urgently needed. Human residents of endemic areas develop protective immunity that limits parasitemia and disease, and naturally acquired human immunity provides an attractive model for novel vaccine antigen identification. As part of the MOMS project, 785 Tanzanian children living in an area of intense malaria transmission were enrolled at birth, and intensively monitored for parasitemia and clinical illness for up to 3 years, with an average of 47-blood smears/child. We identified resistant (n=10) and susceptible (n=10) children based on the results of monthly blood smears obtained from the age of 2 to 3 years with matching for potential confounders. Using a differential library screening approach, we identified parasite genes that encode proteins uniquely recognized by plasma pooled from resistant, but not susceptible children. We characterized these candidates with western blot and immunolocalization assays and validated them with independent selections of plasma and with growth inhibition assays. We screened 750,000 clones and identified 3 clones uniquely recognized by resistant but not susceptible plasma. These encoded MSP-7, and hypothetical proteins on chromosomes 10 and 11. We expressed and purified clone 10 and generated anti-sera which, in accordance with in silico predictions, recognized a 244 kDa antigen in P. falciparum infected, but not uninfected RBCs. In inhibition growth assays, anti-clona 2 anti-sera inhibited parasite growth by 48-63% in several parasite strains. In an ELISA assay using independent selections of resistant (n=11) and susceptible (n=14) plasmas, resistant individuals had 4 fold higher antibody levels to clone 2 proteins compared to susceptible individuals. In conclusion, our differential screening approach identified several novel vaccine candidates and we are currently evaluating the relationship between antibody levels to clone 2 and resistance to infection and disease in the entire birth cohort.

1217

A NEW MALARIA EXPERIMENTAL CHALLENGE SYSTEM: INFECTION OF VOLUNTEERS BY THE BITES OF ASEXUAL ANOPELEHS STEPHENSI MOSQUITOES INFECTED WITH PLASMODIUM FALCIPARUM (NF54) SPOROZOITES

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Experimental malaria sporozoite challenge is an essential component of the vaccine development plan for malaria vaccine candidates targeting pre-erythrocytic stages of the parasite. The current challenge model requires the bites of five infected mosquitoes raised in traditional insectaries to consistently induce malaria. We previously reported on an improved malaria challenge system using the bites of one, three or five asexically-raised mosquitoes in compliance with cGMP and demonstrated that the aseptic model is safe, associated with a precise prepatent period, and transmitted malaria to all six participants bitten by three Anopheles stephensi mosquitoes. As a follow-up study, we evaluated the aseptic model using the bites of three mosquitoes in nineteen additional malaria-naive adults. In total, twenty-five adults aged 18-40 years (mean = 30 years) were bitten by three A. stephensi mosquitoes infected with the NF54 strain of chloroquine-sensitive P. falciparum. The geometric mean sporozoite count detected in challenge mosquitoes was 36.1 x 10^3 (range 6.0-71.1 x 10^3). All twenty-five participants developed microscopy-confirmed peripheral parasitemia, seventeen (68%) on Day 11 post-challenge. The mean prepatent period was 10.9 days (range 9-14 days). The mean parasitemia at diagnosis was 10.8 parasites/µL (range 2-44). No serious adverse events were encountered. The most common solicited events included headache, chills, myalgia, and fever. The aseptic, cGMP-compliant challenge model using three infected mosquitoes transmitted malaria to 100% of participants. The cGMP system provides reliable infection and improves the challenge model by establishing a foundation for assessing the infectivity of sporozoites from aseptic mosquitoes after they have been extracted, purified, vialled, cryopreserved, thawed, and administered by needle and syringe.

1218

PHASE 1 STUDY OF THE SAFETY AND IMMUNOGENICITY OF BSAM-2/ALHYDROGEL®+CPG 7909, AN ASEXUAL BLOOD STAGE VACCINE FOR PLASMODIUM FALCIPARUM MALARIA IN ADULTS IN MALI

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A single blind, randomized, controlled Phase 1 clinical trial is being conducted to assess the safety and immunogenicity in malaria exposed adults of the Plasmodium falciparum blood stage vaccine BSAM-2, containing a four recombinant protein mixture of AMA1 (AMA1- FVO+AMA1-3D7) and MSP142 (MSP142-FVO+MSP142-3D7) /Alhydrogel® with the novel adjuvant CPG 7909. Participants are healthy adults 18-45 years old living in the village of Bancoumana, Mali. Thirty participants
have received 3 doses (Days 0, 56, and 120) of either BSAM-2 or Euvax B Hepatitis B vaccine and followed actively for 8 months after the last vaccination and passively for an additional of about 2 months. Enrollment and first vaccinations occurred in March and April of 2010. Vaccinations were well tolerated, with related adverse events being mostly mild or moderate injection site reactions. Antibody responses for AMA1 and MSP1α were higher in the group receiving BSAM-2 for all time points after the first vaccination and the differences were statistically significant (p<0.05). There was no significant increase in antibody levels 14 days after the third vaccination compared to 14 days after the second vaccination. The incidence rate of clinical malaria was similar between the vaccination and comparator groups. Despite the favorable safety profile and good immunogenicity, no further clinical development of BSAM2/Alhydrogel+CPG 7909 is currently anticipated.

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OPTIMIZATION OF A MOUSE CHALLENGE MODEL TO EVALUATE THE EFFICACY OF PLASMODIUM FALCIPARUM CIRCUMSPOROZOITE PROTEIN BASED MALARIA VACCINES

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Future improvements in the protective efficacy of Circumsporozoite protein based vaccines will depend on preclinical data comparing delivery platforms and mixed antigen combinations. Transgenic rodent parasites where the native CSP has been replaced with a functional PICSP gene will be important tools for down-selecting vaccine candidates. We obtained a transgenic rodent parasite line in which the full-length PICSP gene was inserted into the Plasmodium berghei genome, as reported previously. The parasite was adapted to grow at the WRAIR entomology laboratory using serial passages of sporozoite induced and blood induced infections. We confirmed the transgenic nature of the parasite by IFA with species-specific monoclonal antibodies to CS. We observed normal oocyst development, but significantly reduced salivary gland sporozoite burden in mosquitoes. The minimum infective dose of the sporozoites was established and a series of challenge experiments were conducted comparing several PICSP based protein vaccine candidates. Protection was defined as complete absence of blood stage parasites on day 15 post challenge. Using a challenge model to down-select vaccine candidates can have important implications for improving CSP based vaccine candidates.

1220

A NOVEL TRICK TO CONTROL MALARIA: MANIPULATING THE MOSQUITO INNATE IMMUNE RESPONSE AGAINST PLASMODIUM PARASITES TO BLOCK TRANSMISSION

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Malaria is caused by the protozoan parasite Plasmodium which is transmitted by the female Anopheles mosquito. The parasite must complete its sexual development in the mosquito before it can be transmitted to the human host. The innate immune response of the mosquito considerably hinders the development of the parasite but this is often not sufficient to clear the infection. In natural infection of the mosquito by Plasmodium, there has to be a fine balance between the immune response against the parasite and immune pathology which is reportedly detrimental to the health of the mosquito. We have tried to tip this balance in favour of the mosquito’s immune system, which will hinder parasite development and reduce malaria transmission. We have used a viral vectored vaccine platform to express candidate antigens, which are components of the mosquito’s innate signalling pathways. Mice have been vaccinated and serum used to measure transmission-blocking activity of antibodies generated by immunization using standardized readouts of in vivo efficacy and effect on mosquito survival. This novel strategy could be a revolutionary breakthrough as it would not only work against potentially all four malaria species that infect humans, but likely also against some other mosquito-transmitted diseases and could have a major impact in decreasing the burden of vector-borne diseases. We have also used this vaccine platform to screen several leading parasite and mosquito based malaria transmission blocking vaccine candidates The significance of this work is to provide the first and much needed head-to-head assessment of the in vivo efficacy of the known leading TBV candidate antigens, as well as look for novel antigens aimed at de-regulating the mosquito’s innate immune system in favour of transmission-blocking activity.

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ANTENATAL MALARIA AND HELMINTH INFECTIONS ARE ASSOCIATED WITH IMPAIRED VACCINE EFFICACY IN KENYAN INFANTS

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African pregnant women are often chronically infected with parasites whose soluble products can cross the placenta and prime or induce immunomodulatory responses in the fetus that can persist into infancy and could affect infant immune responses to childhood vaccines. To test this hypothesis we examined the effect of malaria, schistosomiasis, and intestinal parasites in pregnant Kenyan women (n=545) on the development of IgG antibody responses to tetanus, diphtheria, hepatitis B virus, Haemophilus influenzae type B (Hib), and poliomyelitis in their offspring following vaccination at 6, 12, 18, 24, 30 and 36 months of age. Overall 64.2% of the pregnant women were infected with helminths: 46% and 18% with single and multiple infections respectively. 29%, 20%, 15% and 10% were infected with schistosomiasis, hookworm, or malaria respectively. Children of mothers infected with malaria had lower diphtheria titers at 6, 12 and 18 months of age as compared to children of uninfected mothers (P<0.01-0.0009 at each time point estimated by generalized estimating equations). Similarly, offspring of schistosomiasis-infected versus uninfected women had lower diphtheria titers at 12 and 24 months of age (P<0.01). In contrast, offspring of schistosomiasis-infected compared to uninfected women had higher polio titers at 12, 18 and 24 months of age (P<0.01 at each time point). Children of mothers infected with 2 or more infections had significantly lower Hib-IgG levels at 12 months of age and higher polio-IgG levels at 18 months of age compared to children of mothers with single infection (P<0.01). There was no significant difference in antibody levels to any childhood vaccines in children of mothers infected with hookworm, Trichuris, or other intestinal helminths as compared to children of uninfected mothers. Thus, malaria and chronic helminth infections during pregnancy alters responses antibody responses to childhood vaccines and highlight the importance of national programs to eradicate malaria and helminth infections in pregnant women.
We have shown that Cry1Ac induces protection against Plasmodium chabaudi AS and *P. berghei* ANKA infection. In this work, we analyzed whether the coadministration of Cry1Ac prototoxin with *P. berghei* ANKA antigen (Ag) potentiates this protection and if oxidative stress is associated to parasite elimination. Groups of CBA/Ca mice were weekly treated with: PBS, prototoxin Cry1Ac, Ag plus PBS or Ag plus Cry1Ac (Ag+Cry) during 5 weeks, one day after the last injection, mice were infected with *P. berghei* ANKA. Parasitaemia, body weight and survival were recorded daily. In addition, on day 9 post infection splenic mRNA was isolated retrotranscribed and analysed for IFN-γ using qPCR, nitric oxide serum levels and catalase activity also were studied. Mice treated with Ag increased survival for 5 days while mice injected with Ag+Cry survived 8 days more compared to mice treated with PBS (control group), both groups of mice treated with Ag developed lower parasitaemias and lower spleen index compared to control group, furthermore, IFN-γ mRNA expression was upregulated, which implies that with lower cell proliferation the better parasite elimination was attained. Mice treated with Ag+Cry developed significantly higher levels of NO and catalase specific activity in the spleen compared to control group, all these results suggest that Cry1Ac prototoxin could be a potential adjuvant for a malaria vaccine.

**NOVEL APPROACH FOR THE IDENTIFICATION OF NATURAL IMMUNE BOOSTING TRANSMISSION-BLOCKING VACCINE AGAINST PLASMODIUM FALCIPARUM**

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Antibodies recognizing the surface of Plasmodium falciparum zygotes and ookinetes are thought to be ideal for the immunological interruption of malaria parasite transmission from vertebrate host to mosquito. After primary vaccination, antibody responses to such antigens would be boosted during infection. Such an approach would have a advantage over current lead TBV candidates such as PfS25 that do not naturally induce immune responses in humans because of very low or lack expression in the human host and/or low antigenicity. Here we propose that the identification of antigens shared between gametocytes, sporozoite and ookinete is a new approach to the development of transmission-blocking vaccines (TBV). We hypothesized that highly expressed ookinete surface proteins of *P. falciparum* that are also expressed by asexuals, gametocytes or sporozoites would boost transmission-blocking antibodies during natural malaria infection. To test this hypothesis, we used existing ookinete proteome data and a *P. falciparum* protein microarray to identify antigens that might boost transmission-blocking activity by being shared among blood and mosquito midgut stages. 110 African patient sera recognized 79 predicted ookinete surface proteins of *P. falciparum* (*P. gallinaceum* ookinete orthologs) on the protein microarray; several ookinete surface proteins were found also to be expressed in gametocyte, sporozoite or asexual blood stage parasites. The hypothetical PF11_0055 gene product contains a predicted thioeredoxin-like domain, is highly conserved (79%) in *P. berghei*, is expressed in all stages, and was immunogenic. Vaccination of mice with recombinant E. coli-produced PF11_0055, followed by *P. falciparum* gametocyte lysate, boosted anti-PF11_0055 antibody titer compared to gametocyte lysate alone used as vaccine. In standard membrane feeding assays, antibodies to PF11_0055 antibodies significantly reduced oocyst numbers and infected mosquito prevalence. Another protein, PFCelTOS, a known sporozoite- and ookinete-expressed protein, was found to be abundant in ookinetes and highly immunogenic (spec count: ookinete 84, sporozoite 58; geometric mean titer 6796); polyclonal mice sera effectively blocked oocyst development in *P. falciparum*. This new approach to transmission-blocking vaccine candidate discovery based on systems biology antigen discovery is a promising new direction in malaria vaccinology.

**LANDSCAPE OF RESPONSIBILITY: EVOLVING OF ROLES AND RESPONSIBILITIES FOR COMMUNITIES AND INSTITUTIONS IN A LARVAL CONTROL PROGRAM FOR MALARIA PREVENTION IN URBAN DAR ES SALAAM, TANZANIA**

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In the first half of the 20th century, targeting the larvae of mosquitoes was regarded as a practical means to reduce malaria in cities. However, because it is labour intensive and demands considerable manpower, entomological expertise as well as institutional oversight, this approach fell out of favour for decades and is only recently being reconsidered.
This analysis describes a community-based programme for larval control of malaria vector mosquitoes in urban Dar es Salaam, Tanzania, as an example of how scientific research and public health governance can be mutually configured in a contemporary African city. Initiated by the Dar es Salaam City Council, the Urban Malaria Control Program (UMCP) was designed to investigate the effectiveness of community-based systems for applying microbial larvicides, to aquatic breeding habitats in reducing the prevalence of malaria. The UMCP aims to demonstrate the operational feasibility of integrating larval control into routine municipal services, relying exclusively for its implementation on community-owned resource personnel (CORPs). The UMCP was therefore, designed to transform Dar es Salaam into both a venue of local management and a site of knowledge production. Drawing on ethnographic and historical resources, we consider the socio-technical practices these parallel transformations entail. In particular, we are concerned with how ‘participation in’ and ‘responsibility for’ larval control is inter-articulated through scientific protocols, development practices, and the specific political history of Tanzania. Through an analysis of the activities of the CORPs, we suggest that public health governance should be understood within a series of partial and spatially-bound relationships: between residents, local government from neighbourhood to city level research institutions and the reproduction traits of specific mosquito species. We conclude that to enable scaling up of a community-based intervention to a sustainably effective programme at city or national level requires, first, attention to the political history of those relationships and, second, an understanding of how responsibility for malaria control and public health more broadly, is best distributed within the simultaneous contexts of a scientific evaluation and a government-led programme.

**PHYSICAL DURABILITY OF TWO TYPES OF LONG-LASTING INSECTICIDAL NETS (LLINS) AFTER TWO YEARS OF USE, MOZAMBIQUE 2008-2010**

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Understanding the physical durability (PD) of long-lasting insecticidal nets (LLINs) is critical to guide malaria programs on the frequency of LLIN replacement. We conducted a prospective evaluation of LLIN PD after a distribution campaign in October 2008 in Nampula Province, Mozambique. During the LLIN campaign we tagged 1000 LLINs of two types (polyethylene [PT] and polyester [PS]) at six distribution sites (6000 LLINs tagged). The tagged LLINs were geo-located during a house-to-house survey one month after the campaign and a random sample of households (HHS) was selected. One and two years after the campaign, the selected HHS were surveyed and all tagged LLINs were collected. LLINs were stretched over a frame against a black background and all holes were quantified. The difference in total number of holes by LLIN type and year was analyzed by unadjusted chi-square and the median number of holes and inter-quartile range (IQR) by hole size was analyzed using Wilcoxon rank sum test. One year after distribution 164 out of 210 HHS were interviewed and 148 LLINs were recovered and assessed; 50 of 51 (98%) PT and 73 of 97 (75%) PS had at least one hole (p<0.0004). Two years after distribution, 197 out of 240 HHS were interviewed and 163 LLINs were recovered; 58 of 59 (98%) PT and 97 of 104 (93%) PS had at least one hole (p = 0.15). The median number and IQR of holes after one and two years of use, respectively, was 18 (9, 33) and 53 (28, 98) for PT and 4 (1, 12) and 15 (5, 45) for PS. For both years, PT had a statistically significant higher number of holes of all sizes compared to PS (p < 0.0001). We found significant proportions of LLINs are damaged already by year one, more so for PT than PS. How this damage to LLINs translates into loss of protection against malaria transmission is not yet known. Additional studies are needed to measure the impact of the number and size of holes and physical integrity of the LLINs on malaria transmission to define LLIN failure.

**FREE NET DISTRIBUTION: WILL A HANG-UP CAMPAIGN MAKE AN IMPACT ON USE?**

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Insecticide treated nets (ITNs) are highly effective in reducing malaria morbidity and mortality when used appropriately and consistently. The Angolan Ministry of Health (MoH) recently revised its National Strategic Plan for 2011-2015 to expand ITN coverage beyond pregnant women and children under five to universal coverage. An effective method to reach and maintain high net coverage is free distribution campaigns. Post-distribution hang-up campaigns to assist in and ensure utilization of ITNs have been implemented in several sub-Saharan countries. Survey results to evaluate the effectiveness of these campaigns indicate higher use of ITNs. In Angola, the first major free net distribution campaign targeting universal coverage is currently underway (April-August 2011). Africare is implementing the campaign in thirty-two communities in four municipalities in two provinces. 176,000 ITNs will be distributed to reach universal coverage in these communities. Door-to-door registration confirms household size, the number of existing ITNs, and the number of additional nets required to ensure each household member has access to an ITN. Vouchers for free ITNs are distributed at the time of the door-to-door registration and are redeemed two weeks later at a central distribution location. Distribution is complemented by community awareness and education activities around malaria prevention and transmission. Activities include demonstrations of how to properly hang and care for ITNs. In two of the four municipalities, a hang-up campaign is being conducted in which community activists visit all households to assist hanging the nets in sleeping spaces. A post-campaign survey to assess ITN coverage and usage is planned for August 2011. Based on interim data collected, a higher use rate is expected in the two municipalities receiving the hang-up campaign compared to those not receiving this intervention. This campaign is important as it will illuminate important barriers, challenges and opportunities that Angola’s MoH can then use to design effective programming to achieve its goal of universal ITN coverage.

**LOW COST REPELLENTS FOR MALARIA PREVENTION IN RURAL AFRICA: THE JURY IS STILL OUT**

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Malaria control using Long Lasting Insecticidal Nets (LLINs) is a highly effective strategy for rural Africa. However, there is growing evidence that malaria vectors are switching their feeding behavior to the early evening when people are not under their nets and are available to feed on. A cluster randomized controlled clinical trial was conducted in a village in Southern Tanzania from June 2009 to September 2010 to evaluate the additional protection provided by a 15% deet (di-ethyl toluamide) repellent lotion among LLIN users compared to LLIN users given a lotion with no deet. Consistent repellent use in the early evening may provide protection from clinical malaria episodes transmitted by early evening feeding mosquitoes. However, the power of this study was insufficiently low to draw a firm conclusion from the data. The estimate protective efficacy was 13%, lower that expected. In order to measure this effect with sufficient power a sample size of more than 5,000 households per arm would be required. The role of repellents in malaria prevention
remains uncertain. Although there were 13% fewer clinical malaria episodes among repellent users compared to the placebo this difference did not reach statistical significance and in order to be sure that repellents are protective a much larger trial would have to be carried out. Repellents were extremely popular and the relief from nuisance biting mosquitoes was a major motivation for their use. They would need to be cheap in order to encourage uptake and strategies such as seasonal promotion prior to peak malaria season could be employed in order to maximize their potential for protection from malaria.

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IMPORTANCE OF SLEEPING ARRANGEMENT TO INCREASE BED NET USE AND REDUCE MALARIA TRANSMISSION

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A previous study found that older children tend to sleep on the living room floor without mosquito nets in villages along Lake Victoria, western Kenya. The study suggested that it is not easy for children to hang a net in a living room. We examined if this situation increases malaria transmission. A total of 849 children less than ten years old were tested for malaria infection using rapid diagnostic tests (RDT). Their caretakers were asked about bed nets use and sleeping arrangement. Of them, 530 children (62.4%) were tested positive. Nearly 70% of them did not sleep on beds, and almost half of them did not use bed nets. Older children more likely slept on the living room floor. Bed net use was lower among older children, and among children who slept on the floor. Children who slept without nets had a higher positive rate for malaria infection. Older children had a higher positive rate. When the analysis was limited to children above five years old, the result of RDT was not significantly correlated with bed net use and sleeping arrangement. The positive rate of older children was 68.7%, while that of younger children was 57.1%. These results suggest that sleeping arrangement is particularly important for younger children to prevent malaria infection.

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SOLAR-POWERED FAN PROVIDES VENTILATION WHILE SLEEPING UNDER INSECTICIDE TREATED BED NETS

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Insecticide-treated bed nets have been shown to reduce transmission of malaria by 50% in numerous endemic regions. However, many recipients are not using their bed nets due to uncomfortably hot conditions while sleeping inside of them. Here we have developed a prototype solar rechargeable fan that can be easily positioned inside the enclosed bed net space to provide ventilation and cool off the occupants. The fan features a self-contained battery pack, motor, switch, and charging circuit that allows the fan to be plugged into the separate solar panel power source. The objective is six hours of exposure to sunlight charges to the battery pack to enable 8 hours of constant operation. The constructed prototype is a proof of concept to show that it is feasible to create a small, efficient solar powered fan. Refinements to the existing prototype will include an alternative battery pack to reduce costs, and design modifications to decrease charging time and to increase air circulation and handling.
Dr Ralph Rack, Avotiana Rakotomanga, Gilles Rebours, Harilala Ranaivohariminina, Marthe Rahelimalala, Allison Belemvire, Donald W. Dickerson, Teresa Bernhard, Alyssa M. Finlay

**SUSTAINABLE SUPPLY CHAINS: LESSONS LEARNED FROM A LONG LASTING INSECTICIDAL NET RECYCLING PILOT PROJECT IN MADAGASCAR**

Distribution, use and timely replacement of long-lasting insecticide treated nets (LLINs) are part of a key malaria prevention strategy in Madagascar, where 5.2 million LLNs were distributed from 2005-2007. There is a growing awareness of the potential environmental impact of insecticide-embedded plastic waste from the increased number of LLNs, if not disposed of or recycled in an environmentally sound manner. We conducted a pilot project to collect and recycle existing old, expired LLNs (oLLNs) in conjunction with a mass free LLN distribution campaign in November, 2010. Six health districts with an estimated population of 1.6 million were targeted for the pilot where 279,000 bed nets had been distributed in 2007. Health volunteers were trained to educate their communities, using a pre-tested job-aid, to voluntarily bring unwanted oLLNs for disposal to the closest campaign community distribution point at the time of collecting their new free LLNs. oLLNs were collected, transported, sorted, compacted, baled and shipped to a plastics recycling company for processing. Over 22,500 oLLNs were collected from 394 out of 489 (81%) community collection points. Of these, 90% were collected post-campaign. Community members were more willing to give up oLLNs once the new LLNs were installed in homes after the campaign distribution. Families with an insufficient number of new nets, and those using oLLNs for other purposes, were reluctant to give up their oLLNs. Sites with the most complex transport logistics were less likely to successfully collect oLLNs. Post hoc radio messaging was found to be a useful tool to reinforce messages. The cost was $2.72/oLLN collected. Costs could be substantially reduced by combining training with other LN distribution campaign preparation activities. LLINs have been successfully recycled and the material is being analyzed and tested for the most appropriate recycling use. In conclusion, collection and recycling of oLLNs was found to be acceptable and feasible. Malaria programs and international donors should further explore and implement cost-effective recycling and re-use options.
because “there are many mosquitoes in our community” or because “there is much dengue and hemorrhagic dengue around”. Though overall levels of concern were low, the main one expressed related to potential allergic reactions to the ITC, particularly among children. Through the FGD we also assessed the style of curtain that people might prefer (lacy ITC was favored by all over simple bednet style because it was “more elegant”), the colors favored (light colors were preferred for most spaces, except where people would use the curtain for additional privacy), and the number of curtains people might request (median number requested was 5). The information obtained allowed us to obtain an appropriate amount and color combination of ITCs for the initiation of the trial. Also, we developed a tri-fold describing the purpose of the study, the ITCs, and providing information on how to care for the curtains, making sure to incorporate the types of concerns expressed during the focus groups. Formative research allowed us to obtain information in a rapid and cost-effective manner that was useful for the start up of our trial.

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CO-OCCURRENCE PATTERNS OF THE DENGUE VECTOR AEDES AEGYPTI AND AE. MEDIOVITTATUS, A POTENTIAL NATIVE DENGUE VECTOR IN PUERTO RICO

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Aedes aegypti is implicated in dengue transmission in tropical and subtropical urban areas around the world. Ae. aegypti populations are controlled through integrative vector management. However the efficacy of vector control may be undermined by the presence of alternative, competent mosquito species. In Puerto Rico, a native mosquito, Ae. mediovittatus, is a competent dengue vector in laboratory settings and it spatially overlaps with Ae. aegypti. It has been proposed that Ae. mediovittatus may act as a dengue reservoir during interepidemic periods, perpetuating endemic dengue transmission in rural Puerto Rico. Dengue transmission dynamics may therefore be influenced by the spatial overlap of Ae mediovittatus, Ae. aegypti, dengue viruses, and humans. We take a landscape epidemiology approach to examine the association between landscape composition and configuration and the distribution of each of these Aedes species and their co-occurrence. We used remotely-sensed data from a newly launched satellite to map landscape features at very high spatial resolution. We found that the distribution of Ae. aegypti is positively predicted by urban/built-up density and by the number of tree patches, Ae. mediovittatus is positively predicted by the number of tree patches, but negatively predicted by large contiguous urban/built-up areas, and both species are predicted by urban/built-up density and the number of tree patches. This analysis provides evidence that landscape composition and configuration is a surrogate for mosquito community composition, and suggests that mapping landscape structure can be used to inform vector control efforts as well as to inform urban planning.

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Biodiversity of mosquitoes (Diptera: Culicidae) and sand flies (Diptera: Phlebotominae) from the northwest region of Loreto department in Peru

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From January to March 2009, mosquitoes and sand flies were collected in four villages located on the margins of the Huallaga and Maranon Rivers, in the provinces of Alto Amazonas and Datem del Marañon, located in Loreto Department, Peru. Collections were made using CDC light traps, human bait, and back-pack aspira tors in peri-domestic areas. The entomologic material was kept in liquid nitrogen and transported to the Entomology Lab of NMRCID in Lima, where taxonomic identification was carried by mosquitoes and sand flies. A total of 22,513 mosquitoes were identified: 21,899 (97.27%) Culicinae and 614 (2.71%) Anophelinae. Mosquito capture rates were 75.28% using CDC light traps, and 17.27% using human bait, and 7.45% using back-pack aspirators. Throughout the process after collection (transport, storage, taxonomic identification), mosquitoes were preserved in cryovials at a temperature of -80°C. Biodiversity rates of Anopheles spp. subgenera Anopheles, Nyssorhynchus and S tethomyia were determined. Anopheles (Nyssorhynchus) spp. had the highest density in all collections. Eleven genera of Culicinae were identified, the Culex genus (with two subgenera and about 10 species identified) had the highest number of collected mosquitoes, followed by the genera Mansonia, Ochlerotatus, Psorophora and Coquillettidia. The Shannon-Weaver diversity index was high with CDC light traps (H = 1.04), in relation to the other collection methods. In relation to sand flies, 113 specimens of the genus Lutzomyia (77 females and 36 males) were identified, with 11 species and three Lutzomyia spp., from which Lutzomyia (Nyssomyia) antunesi had the largest number of collections (64 sand flies), followed by Lutzomyia (Nys.) yuilli yuilli (14).

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Differential expression of Aedes aegypti salivary proteome upon Chikungunya virus infection

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Mosquito-borne diseases are excellent examples of emerging and resurging diseases that are significant global public health threats. Chikungunya virus (CHIKV) infection caused an explosive outbreak that infected as many as two million people during 2006 in India and the islands of the Indian Ocean with subsequent spread to other parts of the world. This resurging infection is transmitted primarily by Aedes aegypti and Ae. albopictus. Saliva of Ae. aegypti contains a complex array
of proteins essential for both successful blood feeding and pathogen transmission. Understanding salivary gland protein expression during the extrinsic incubation period of CHIKV infection is important since changes in salivary gland physiology and saliva composition could influence mosquito blood feeding success and virus transmission. CHIKV regulated mosquito salivary proteins could modulate host innate and acquired immune responses at the bite site and systemically, resulting in impaired antiviral effector functions. Using a differential proteomic approach we investigated the differential mosquito salivary protein expression during CHIKV infection. Adult female mosquitoes were fed with either CHIKV infected or uninfected bovine blood using a Hemotek membrane feeding system. Salivary glands were dissected eight days postfeeding, and proteins were extracted in 2D gel buffer. One hundred micrograms of proteins were resolved on a 2D-gel and stained with SYPRO-Ruby stain. Protein spots with a relative difference of greater than two fold, and a p-value less than 0.05 were considered a significant variation. These protein spots were excised, tryptic digested and prepared for MALDI-TOF-TOF and LC-MS-MS analysis. The expression of 22 proteins was found to be up-regulated, while 33 proteins were down-regulated. Among the up-regulated proteins, adenosine deaminase and D7 proteins have been implicated to play a major role in mosquito blood feeding. The D7 proteins belong to the family of arthropod odorant binding proteins, that facilitate blood feeding by binding to biogenic amines. These proteins are believed have anti-hemostatic and anti-inflammatory functions. Interestingly, several of the differentially expressed proteins in the salivary gland induced by CHIKV infection are proteins with unknown functions. This preliminary data establishes that CHIKV modulates mosquito salivary gland protein expression.

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BLOODFEEDING PATTERNS OF CULEX TARSALIS AND THE CX. PIPIENS COMPLEX IN CALIFORNIA

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West Nile virus (WNV) is a mosquito-borne flavivirus now endemic across several ecological regions in California. These regions are home to a wide diversity of potential avian and mammalian hosts as well Culex vector species. Because avian hosts have varying WNV competence, determining the bloodfeeding patterns of the Culex vectors is important in understanding the dynamics of virus maintenance as well as incidental transmission to disease-susceptible humans and horses. The bloodfeeding patterns of Cx. tarsalis and members of the Cx. pipiens complex were investigated from 5 locations spanning over 850km from Northern to Southern California. Nearly 100 different avian, mammalian and reptilian host species were identified from 1,487 bloodmeals using DNA sequence from a portion of the mitochondrial gene, cytochrome c oxidase I (COI). Cx. tarsalis fed on a higher diversity of hosts and more frequently on non-human mammals than did members of the Cx. pipiens complex when collected in the same area. Several WNV competent avian species, including House Finch and House Sparrow, were common bloodmeal sources for both vector species across several ecological regions and could account for WNV maintenance, particularly in urban settings. Highly competent Western Scrub-Jay, Yellow-billed Magpie, and American Crow also were fed upon frequently when available and are likely important amplifying hosts in some areas. The Cx. pipiens complex (0.4%) fed more frequently on humans than did Cx. tarsalis (0.2%), and horse bloodmeals were only identified from Cx. tarsalis (2.3%). Although neither vector species fed frequently on humans or horses in this study, with high vector abundance both species could serve as bridge vectors of WNV in several California regions.

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BARRIERS TO MALARIA ELIMINATION ON THE ISLANDS OF ZANZIBAR

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The islands of Zanzibar are the major focus of a malaria elimination campaign (defined as the reduction to zero of the incidence of locally acquired malaria). It is generally supposed that P. falciparum in Zanzibar is vectored by Anopheles species that are endophilic, anthropophagic and pyrethroid-susceptible. As a result, the islands have been saturated with permethrin or alphacypermethrin treated LLINs (long lasting insecticide treated nets) and IRS (indoor residual spraying) with lambdacyhalothrin. These campaigns have been extremely effective at reducing the prevalence of malaria to less than 1 percent. It now seems however, that the move to an elimination stage will be complicated by some recent discoveries on the ecology and behavior of local mosquito populations. Studies conducted on the island of Pemba by the Zanzibar Malaria Control Program during 2010 and 2011, now show that most of the remaining transmission in Pemba is probably being mediated by An. arabiensis, and that (as a consequence of the behavioral plasticity of that species, and the high coverage of pyrethroids indoors) the majority of bites are now received out-of-door. This suggests that LLINs and IRS may need to be augmented by other control methods in order to reduce mosquito-human contact further. Moreover, a phenological characterisation of An. arabiensis from Pemba have shown these populations to be resistant to all pyrethroids (but susceptible to DDT, malathion and bendiocarb). The magnitude of the resistance is sufficient to markedly reduce mortality in simple bioassays against IRS residues, and used LLINs. This has prompted ZMCP and its partners to implement a change in IRS practice but with so few new vector control interventions available, or even in the pipeline, opportunities to improve upon existing control practices are very limited.

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NOVEL SOLUTIONS FOR THE DETECTION, PREVENTION AND TREATMENT OF VECTOR-BORNE DISEASES

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Florida’s Schools of Pharmacy at UF and USF in concert with the UF-Emerging Pathogens Institute (EPI) and USF Center for Drug Discovery and Innovation (CDDI) and Global Health Infectious Disease Research Program (GHIDR) are developing a consortium for novel solutions for the Detection, Prevention and Treatment of Vector Borne Diseases. Vector borne diseases represent a significant health care challenge for Florida (and the tropical world), but there has been little economic incentive for the pharmaceutical industry to develop interventions. Our proposed consortium is critical to catalyze the development of efficient strategies able to solve this regional/global health-care challenge. The proposed consortium will provide a “case study” to introduce the FDA’s Critical Path Initiative Development Toolkit to Florida institutions, with a focus on developing powerful scientific and technical methods such as in vitro, animal or computer-based predictive models, biomarkers for safety and effectiveness and new clinical evaluation techniques for a streamlined and efficient drug development and as well for establishing new validated
methods of detection and prevention. This new USF-UF consortium will place emphasis on product innovation and translational medicine and allow students and faculty to participate as team members in high profile epidemiological, drug discovery and development projects. Our consortium will "pull" and our Centers and Institutes will "push" the best emerging biomedical and biopharmaceutical technologies in Florida. Resulting infrastructure will facilitate faculty scholarship and intellectual engagement between our Universities and business and economic constituencies throughout the state and nation.

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COST-EFFECTIVE COLLABORATION BETWEEN THE UNITED STATES AND PERUVIAN NAVIES AND A PERUVIAN UNIVERSITY TO PROVIDE IMPROVED PUBLIC HEALTH MEASURES AGAINST DENGUE AND YELLOW FEVER IN PERU

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In Peru, there are no formal medical entomology programs available at the graduate or post-graduate, and very limited training at technical levels. However, Peru is endemic to many medically-important insects, including *Aedes aegypti*, which vectors dengue and yellow fever virus pathogens into human populations. An international collaboration was formed between the Peruvian Instituto de Medicina Tropical “Daniel A. Carrion” of San Marcos University (IMT DAC UNMISP), 1 the Entomology Department of the United States Naval Medical Research Unit No. 6 (NAMRU-6), and the Sistema de Alerta DISAMAR of the Peruvian Navy Clinic. This collaboration resulted in the provision of formal medical entomology training specifically focused upon surveillance and control of *Aedes aegypti*, the mosquito vector of dengue and yellow fever viruses, to Peruvian naval Nurses, who will be stationed in remote locations throughout Peru during their Naval careers. This collaboration has been organized as a long-term collaboration, with the goal of providing this training 2-3 times each year to new active-duty Peruvian nurses prior to their deployment to remote areas in Peru that are endemic to these debilitating diseases.

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CHANGES IN RELATIVE ABUNDANCE OF *ANOPOHELIS GAMBIAE* S.S. AND *AN. ARABIENSIS* IN SUBA DISTRICT, WESTERN KENYA: ITS RELATION TO BED NET COVERAGE

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Coverage of insecticide treated bed net has increased considerably in Kenya for the past few years. Since insecticide treated nets kill indoor mosquitoes, the relative abundance of *Anopheles gambiae* s.s. to *An. arabiensis* may decrease, because *An. gambiae* is more endophilic and anthropophilic. We compared the current relative abundance of both species with that in the past in Suba District. Then, we examined the relationships between relative abundance and bed net coverage. Anopheline larvae were collected from the same areas in 2009 and 2010 that were surveyed by a study in 1998. Indoor resting anophelines were also collected in the same villages in 1999 and 2008. Moreover, we monitored the relative abundance and bed net coverage periodically from 2007 for three years. In the larval survey, over 90% of collected larvae were *An. arabiensis* in 2009 and 2010 while approximately 70% were this species in 1998. The density of indoor resting anophelines in 2008 was one seventh of that in 1998. The decrease was mainly due to the decrease of *An. gambiae* s.s., which increased the relative abundance of *An. arabiensis* from 9.3% to 39.2%. The three-year survey revealed non-linear relationships between bed net coverage and relative abundance of *An. arabiensis*. When coverage exceeded 0.7 nets per person, the density of *An. gambiae* s.s. decreased, and the relative abundance of *An. arabiensis* increased. However, the trend was unclear below 0.7 nets per person.

The results support the notion that bed net coverage alters the relative abundance of malaria vector species. In an area where *An. arabiensis* is dominant, the effectiveness of bed nets may be hampered.

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LA CROSSE ENCEPHALITIS IN EASTERN TENNESSEE: EVIDENCE OF INVASIVE MOSQUITO (*AEDES ALBOPICTUS* AND *OCHLEROTATUS JAPONICUS*) INVOLVEMENT IN THE TRANSMISSION OF AN INDIGENOUS DISEASE

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La Crosse encephalitis virus (LACV), family Bunyaviridae, is an important cause of pediatric encephalitis in the United States. The virus is transmitted by the bite of infectious mosquitoes, primarily the native tree- hole mosquito *Ochlerotatus triseriatus*. Since being characterized in the 1960s, human cases have been concentrated in the upper-Midwestern states where the virus is considered endemic. Approximately 80-100 cases are reported annually. While death is rare, symptoms can be severe and often require hospitalization. In the mid 1990s, a new focus of the disease was recognized in West Virginia, North Carolina and eastern Tennessee. One hypothesis for the establishment of this new focus is that the invasive mosquito, *Aedes albopictus*, may be acting as a novel vector in this area. A third mosquito species, *Oc. japonicus*, has recently become established in the region and is also a competent vector of LAC in the laboratory.

The potential for invasive mosquitoes to modify disease epidemiology is large. These three species occupy many of the same larval habitats and the invasive species may have an effect on the local mosquito community due to resource competition. To test the invasive vector hypothesis, mosquito eggs, larvae, and adults were collected weekly from six recent human case sites in eastern Tennessee from May - August 2010. Three pools of *Ae. albopictus*, one pool of *Oc. japonicus* and eight pools of *Oc. triseriatus* were LACV positive by PCR. Additionally, eleven of the twelve positive pools came from mosquitoes collected as eggs, indicating active transovarial transmission. This is the second study to find field caught mosquitoes positive for LACV in Tennessee with the first sample being *Ae. albopictus* from 1999. To our knowledge, this is the first record of *Oc. japonicus* being naturally infected with LACV and in close association with human habitation. This study provides further evidence that invasive species may have changed the epidemiology of a vector-borne disease in the United States. Viral assays are ongoing.

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INVESTIGATIONS INTO MOSQUITO BLOOD FEEDING PATTERNS ON WILDLIFE AND A POTENTIAL ROLE FOR BATS IN ARBOVIRUS TRANSMISSION CYCLES IN UGANDA

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Zoonotic and vector-borne pathogens have comprised a significant component of emerging human diseases in the last decade. Uganda has a history of enzootic and epizootic arbovirus activity and has been predicted as a hot spot for disease emergence. Serological evidence
exists documenting exposure of various East African bat species to many arboviruses including Rift Valley fever, Yellow fever, West Nile, Usutu, Sindbis, Bunyamwera, and Zika viruses; however, the role of bats in arbovirus transmission cycles is poorly understood. While collecting mosquitoes as part of an emerging arbovirus surveillance project in Uganda, we obtained blood-engorged Culex mosquitoes which had fed on fruit bats in both Semiliki and Maramagamo Forests. To follow up on these observations and investigate the role of bats in arbovirus transmission cycles, blood samples from Rousettus aegyptiacus bats collected from the python cave in Maramagamo Forest were screened for West Nile, Yellow Fever, Dengue, Chikungunya, and O’nyong’nyong viruses by plaque reduction neutralization test (PRNT) and mosquitoes were trapped from around the vicinity of the cave. Blood and tissue samples were also collected from various fruit and insectivorous bat species in Kampala, Uganda and tested for evidence of arbovirus infection by PRNT and virus isolation. Serological and virological evidence will be presented on the arbovirus exposure history of several species of bats in Uganda. The blood feeding patterns of mosquitoes on a diversity of wildlife species in Uganda and potential enzootic arbovirus transmission cycles between mosquitoes and wild vertebrates including bats will be discussed.

LANDSCAPE ECOLOGY OF DENGUE AND CHIKUNGUNYA SYLVATIC VECTORS IN SOUTHEASTERN SENEGAL

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Dengue (DENV) and chikungunya viruses (CHIKV) circulate in a sylvatic transmission cycle between non-human primates and arboreal Aedes spp. in Kedougou, Senegal, and several studies have shown a low incidence of infection by both sylvatic viruses in humans in West Africa as well. Although humans are probably infected by sylvatic vectors, the extent and mechanisms of contact between humans and sylvatic vectors remains unknown. To gain insight into the role of different mosquito species in both enzootic transmission in primates as well as spillover into humans, between 2009 and 2010 we monitored the distribution of a broad array of mosquito species in five landscape classes (forests, savannahs, barren, agricultural, and villages) in the Kedougou area. Mosquitoes were collected monthly in each of the landscape classes from 18:00 to 21:00 hrs and identified to species. Among 39,799 mosquitoes collected, the most and least abundant species were Ae. vittatus and Ae. aegypti, respectively. The abundance of Ae. vittatus, Ae. luteocephalus and Ae. aegypti peaked in June, while that of other species peaked twice between July and November, 2009. The preferred habitat of Ae. africanus, Ae. luteocephalus and Ae. aegypti was the forest canopy, while the other species were distributed more evenly across the five landscape classes. CHIKV was detected by real-time PCR assay and/or virus isolation in 39 pools of mosquitoes, including previously recognized (Ae. furcifer, Ae. taylori, Ae. dalzieli, Ae. luteocephalus, Ae. africanus, Ae. aegypti, Ae. neoafricanus, Ae. hisutus, An. funestus, An. coustani, Ma. uniformis) and potentially new (Ae. metallicus, Ae. centropunctatus, Ae. hisutus, An. domicola and Cx. poecilipes) CHIKV vectors. Infection rates showed temporal and spatial variation. No DENV was detected. Our findings provide insight to the ecology of sylvatic vectors of DENV and CHIKV in a changing environment affected by urbanization and deforestation associated in part with mineral exploitation.

EVOLUTIONARY HISTORY OF Aedes aegypti: A GLOBAL PERSPECTIVE

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Aedes aegypti is the principal vector of both dengue and yellow fever viruses worldwide. A human commensal, this mosquito species has successfully invaded much of the tropical and subtropical world over the past few centuries. Though Ae. aegypti is often treated as a homogenous species, populations of the mosquito differ markedly in their association with human habitats, as well as in their ability to transmit dengue viruses. Recent microsatellite work in our lab suggested that the African sylvan subspecies, Ae. aegypti, is ancestral to the worldwide domestic form (Ae. a. aegypti), but that close human association has likely evolved multiple times independently in Ae. aegypti. In order to more formally test hypotheses of ancestry and trait evolution, we sequenced 4 variable nuclear loci from 167 individuals representing 17 global populations of Ae. aegypti. The same regions were sequenced in two closely related species to provide outgroups for rooted phylogenies. In addition, a sequenced RAD (restriction-site associated DNA) approach was undertaken to explore at a fine-scale the history and colonization of Ae. aegypti out of Africa and across the global tropics and sub-tropics. This method allows simultaneous detection and screening of thousands of SNPs across the Ae. aegypti genome. Bar-coded RAD libraries were successfully constructed from 136 individual mosquitoes (8 each from 17 populations) and sequenced on an Illumina platform. Both the 4 sequenced nuclear loci and the RAD markers confirm African Ae. aegypti as the ancestral form of the species, and support multiple “domestication” events. However, the RAD markers are significantly more sensitive at detecting population structure and tracing the invasion history of this important vector arthropod out of Africa and across the world. In addition, the SNPs detected in our RAD analyses will prove useful in future association mapping studies, such as those for important epidemiological traits including vector competence for dengue and human host preference.

HOST ATTRACTION OF ANOPHELES IN SOUTH HALMAHERA, INDONESIA

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The feeding behaviors of Indonesian malaria vectors remain largely uncharacterized. A Latin square design was used to compare anophelines attracted to human, cow, and goat-baited tents. The experiment was carried out for 12 nights in August 2010 in Saketa village in South Halmahera, Indonesia. Specimens were collected from the inside walls of baited tents every hour from 18:00 to 7:00 hours and were morphologically identified. A subset of blooded specimens were analyzed using a bloodmeal diagnostic PCR assay. 1,235 Anopheles specimens of nine different morphological species were collected over 12 catch nights. These morphological species included An. farauti, An. hackeri, An. indefinite, An. kochi, An. punctulatus, An. subpictus, An. tessellatus, An. vagus, and An. vanu, all of which have been previously shown to be capable of transmitting Plasmodium parasites. 1024, 137, and 74 anophelines were collected in cow, goat, and human-baited tents, respectively. Bloodmeal analysis of specimens collected in the human-baited tent indicate a low level of multiple host blood feeding. Morphological species distribution was similar between the cow and goat-baited tents, with a majority (44% and 36%) of An. indefinite,
but different for the human-baited tent, with a majority (41%) of An. vagus. Eight of the nine morphological species represented in this study were captured on each of the three hosts, suggesting a plasticity in host attraction behavior. Multiple host feeding and flexibility in feeding behavior could have important implications for malaria control.

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ASPECTS OF ECOLOGY OF POTENTIAL RIFT VALLEY FEVER VIRUS MOSQUITO VECTORS, KHARTOUM STATE, SUDAN

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Rift valley fever epidemics are disruptive and expensive to local and regional economies. After a devastating outbreak of Rift Valley Fever in Khartoum state, Sudan 2007; ecological baseline surveys were conducted in Khartoum State, Sudan, during the rainy season (end of July to the beginning of September) 2008 in order to identify mosquito species present and evaluate their emergence and survivorship. Larval identification of species of Culicine and Anopheline mosquitoes present in Khartoum State taken from five study sites represents Khartoum state indicated that Anopheles arabiensis is the only species of the Anopheline mosquitoes found. Three species of culicine mosquitoes were found: Culex quinquefasciatus, Cx univittatus and Cx arbeeni, Species of Aedes were found in irrigated schemes at one study site and was absent from the other four study sites, these species were Ae. vittatus and Ae. vexans, whose presence was recorded after the onset of the rainy season the same breeding site was first occupied by Ae. vittatus then Ae. vexans, with an interval of habitat drying. Daily emergent adults Culicine and Anopheline mosquitoes present were taken from randomly selected breeding sites in the five study sites, population measurements were performed. The absolute number of emergent adults was obtained by collecting mosquitoes under net-traps covering the breeding sites. Records were taken each day for seven constitutive days, synchronized emergence of males and females was observed at all the study sites, showing an overall marked predominance of females in emergence trap catches. Adult survival rate was the most important factor determining the stability of the population and total egg production. Females that become infected when taking a blood meal must survive throughout the incubation period of the pathogen. Under controlled laboratory environment, effect of food types (sucrose 10%, sucrose 10% and blood diet) on longevity of adult female mosquitoes was conducted, sugar-fed and blood-fed mosquitoes exhibited very high percentage of surviving rates beyond the 15 days (incubation period for RVFV), However these have varied among the five study areas. We found that tires where the main breeding site followed distance. We found that tires where the main breeding site followed:

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A COMPARATIVE EVALUATION OF SIX DIFFERENT MALARIA VECTOR COLLECTION METHODS IN LOW-LYING MALARIA ENDEMIC REGIONS OF WESTERN KENYA

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Outdoor biting and other forms of behavioral adaptation by malaria vectors to domestic insecticide-based control measures may compromise the sensitivity of conventional sampling tools operating indoors such as light traps or indoor resting catches, thus preventing effective surveillance and management of vector populations. We evaluated six different vector collection methods to optimize a protocol for operational sampling of malaria vectors robust to variations in vector behavior, notably variations associated with the presence of important malaria control methods. Over 30 days, we replicated a Latin square design 10 times at sites in 4 districts in western Kenya: Kisumu, Bondo, Nyando and Rachuonyo. Each site consisted of 3 locally representative houses through which the six different sets of trapping methods were rotated every 3 nights in a random order of three possible arrangements: 1) Indoor human landing catch (HLC) and outdoor HLC, 2) CDC Light trap placed beside an occupied insecticide-treated net indoors combined with Ifakara tent traps outdoors, and 3) Window traps to catch exiting mosquitoes combined with both pot and box formats of resting traps placed both indoors and outdoors. At each site, a fourth house was selected for pyrethrum spray catch (PSC). The top collection methods with their corresponding number of Anopheles per collection effort were PSC (10.5), HLC indoor (3.0), Light trap (3.0) HLC Outdoor (2.8) and Ifakara tent traps (2.7). Resting Boxes and Pots positioned both indoors and outdoors caught less than 1 Anopheles per collection effort. HLC outdoor collected the highest amount of Culex at 77.4 per collection effort. Irrespective of the intensity or type of insecticide-based vector control method in place and of biting behavior of the local malaria vectors, we conclude that pyrethrum spray catch is the most sensitive method for vector collection in low lying malaria endemic regions of western Kenya.

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HETEROGENEOUS FEEDING PATTERNS OF Aedes aegypti in HOUSEHOLDS IN IQUITOS, PERU

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Heterogeneous biting by female mosquitoes can significantly alter transmission of mosquito-borne pathogens. Previous studies show Aedes aegypti, the primary vector of dengue viruses (DENVs), more frequently bite individuals with higher body mass index (BMI). Because BMI increases with age, we expect positive linear relationship with age and biting. Studies show, however, that young adults receive more bites than older adults. Factors such as sex, mosquito exposure time and previous DENV infection should, therefore, be used to analyze heterogeneous feeding patterns. Between October 2009 and November 2010, 2,035 interviews with 280 participants were conducted in 19 households in Iquitos, Peru.
Interviews focused on anthropomorphic characteristics and time spent in houses. In the week following interviews adult mosquitoes were collected twice daily, yielding 1,878 engorged and partially engorged mosquitoes. Engorged abdomens were excised and participant DNA was obtained by cheek swab. All DNA was extracted using Qiagen extraction columns. Human DNA was amplified at 10 microsatellite loci, and allelic profiles identified using capillary electrophoresis. A computer program matched participant profiles to mosquito blood meals. To date, 99 of 115 identified blood meal profiles have been matched to participants. 29 young adults (ages 15 - 35) received 50 bites (1.72 bites/person). 14 children (<15) and 23 older adults (>35) received 154 and 34 bites, respectively (1.07 and 1.48 bites/person). In one household of 12 residents ranging in age from 5 to 70 years with BMI of 13 to 32 kg/m², 2 young adults ages 27 and 31 with BMIs of 23 and 21.2 contributed to 46% of the 26 identified blood meals, consistent with the idea that young adults are bitten most often, and indicating that age better predicts biting frequency than BMI. Analysis of the remaining 1,763 mosquitoes and interview data will be completed in the next 4 months. Results will be used to model virus transmission and to compare various vaccine delivery strategies.

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WHOLE GENOME SEQUENCING OF ANOPHELES PUNCTULATUS SIBLING SPECIES OF PAPUA NEW GUINEA

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The Anopheles punctulatus (AP) group in Papua New Guinea and Southwest Pacific consists of at least 13 sibling species that include the vectors of malaria and lymphatic filariasis. Understanding the population organization of the mosquitoes as well as the molecular basis for the phenotypic variability related to vector competence or control is complicated by limited data on the genetic diversity of these mosquitoes. We present here data generated by whole genome sequencing from individual AP mosquitoes and show that this approach provides extensive catalogues of genetic polymorphisms and can significantly contribute to better understand the biology of these mosquitoes. We extracted DNA from individual mosquitoes, and after determination of the species status by species-specific PCR-based assay, sheared the DNA molecules into 250-300 bp fragments and prepared libraries for two Anopheles punctulatus mosquitoes, one An. farauti 1, one An. farauti 2 and one An. koliensis.

We sequenced each library on individual lanes of an Illumina GAIIx (paired-end 51 bp) or HiSeq 2000 (paired-end 100 bp). Overall, less than 1.5% of the reads generated could be mapped to the An. gambiae (AG) reference genome sequence suggesting that the sequence divergence between AP and AG is too great for the latter to serve as a useful reference sequence. We therefore reconstructed large chromosomal segments (“contigs”) using solely the sequence information contained in the reads. Using this procedure we successfully assembled the entire mitochondrial genome sequence for each of the five mosquitoes which confirmed the deep divergence between AP and AG but also revealed deep divergences among the AP sibling species. In addition, we assembled 50-60% of each genome into fragments larger than 1,000 bp and identified more than 40,000 DNA polymorphisms that can now be used in association studies for traits related to insecticide resistance, preference to human blood meal or capacity to transmit malaria and filariasis.

A COMPUTER SYSTEM FOR FORECASTING WEST NILE VIRUS RISK USING EARTH OBSERVATION DATA

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Although there have been many calls to expand the use of earth observation technologies in the health sciences, there are few examples of operational systems with demonstrated impacts on public health. Our research objective was to bridge the gap between remote sensing and public health by developing decision support systems to provide health scientists and practitioners with access to environmental information for surveillance and forecasting of mosquito-borne diseases. Specific objectives were to automate the processing of remote sensing data to generate environmental metrics, analyze the predictive capabilities of these metrics using retrospective datasets of human disease cases, and develop a web-based system for visualization and analysis of the resulting products. The system was programmed using JAVA for user interface development and overall system control. Spatial analyses were carried out using Python scripts to call ArcGIS geoprocessing functions. PostgreSQL was used for the storage and manipulation of the resulting data summaries. We implemented a prototype of the system to forecast outbreaks of West Nile virus in the northern Great Plains. Environmental variables included MODIS land surface temperature (LST) and vegetation indices (e.g., NDVI, EVI) derived from the MODIS nadir BRDF-adjusted reflectance product. We also used these data to compute actual evapotranspiration (ETa) using the simplified surface energy balance method. Statistical analysis using generalized additive models (GAMs) revealed non-linear associations between interannual variability in WNV incidence and interannual deviations of cumulative LST, NDVI, and ETa throughout the spring and early summer. There was an early-season influence of the timing of spring onset (captured by NDVI) as well as a late spring/summer influence of accumulated moisture and temperature (captured by LST and ETa). Forecasts are currently being disseminated via a web atlas (http://globalmonitoring.sdstate.edu/eastweb) and will be validated using surveillance data from the 2011 WNV season.

DYNAMICS OF ANOPHELES GAMBIAE POPULATIONS IN THE SAHEL: NEW PATTERNS AND NEW PUZZLES AWAIT NEW UNDERSTANDING

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Malaria remains a top public health priority across Sub-Saharan Africa, where it is transmitted primarily by Anopheles gambiae s.s. and An. arabiensis. Populations of these species exploit diverse environments including dry savannahs and semi-desert areas, where surface waters required for larval development are absent for large parts of the year. How mosquitoes survive the long dry season has been debated without resolution for over 60 years. Although recent studies provide evidence for aestivation (extended survival throughout the 4-7 month-long dry season) of M form An. gambiae, the role of long-distance migration from areas with year-round breeding remains unclear. Here, we analyze the dynamics of the members of the An. gambiae complex in the Sahelian village Thierola (Mali), focusing on the dry season and its preceding and subsequent transition periods, over a period of three years (2008-2011). The dry season mosquito populations were characterized by low overall density (<0.05 mosquito/house), and were predominantly composed of...
the M form (>95%), with the remainder being An. arabiensis. Males were found throughout the dry season, both indoors and in swarms, albeit in very low numbers. Interestingly, the dry-season dynamics were not stable: in early April, ~2 months before the first rain, density surged up to three orders of magnitude and neared to typical dry-season density within days. This surge was observed in both 2010 and 2011 and consisted only of the M form. Five to seven days after the first rains (early June), before a new generation of adults could be produced, the M form surged again over one order of magnitude, and continued to increase gradually at an average rate of 50%/week, for several weeks. Unlike the M form, the S form and An. arabiensis remained virtually zero for over four weeks after the first rains; thus it is unlikely that they aestivated but would emerge only ~5 weeks after all larval sites filled. These results suggest that both the S form and An. arabiensis persist in the Sahel primarily by migration whilst the M form aestivate. Final analysis and implications for malaria control will be presented.

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DIRECT AND INDIRECT COSTS OF PLASMODIUM INFECTION ON MOSQUITO REPRODUCTIVE SUCCESS

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Infection with malaria parasites reduces the immediate reproductive success of mosquitoes, but the life-long effects, as well as their interaction with stress, are not well known. Additionally, the negative effects of infection may be exacerbated by the nutritional cost of feeding on anemic blood. We evaluated the effect of Plasmodium gallinaceum infection on reproductive success of stressed and unstressed Aedes albopictus, fed on either infected or uninfected chicken blood. Each of these treatment combinations were subdivided into three subgroups that were either fed: (i) directly on an infected (or uninfected) chicken (Live); (ii) membrane-fed on fresh blood from the same chicken (Mem Live), (iii) membrane-fed on the same blood incubated at 4°C for 12 h (rendering infectious blood non-infectious; Mem Noninf). The mosquitoes were subsequently fed two more times on uninfected blood from the same chicken. The egg batch size (EBS) of individual mosquitoes was determined 7 d after each feed. Preliminary analyses revealed that EBS was lower in infected vs. uninfected and stressed vs. unstressed mosquitoes. However, the interaction between stress and infection was not significant. Likewise, there was no significant interaction between infection and feeding type (i.e. Live, Mem Live and Mem Noninf), indicating that the fitness costs of being fed on an infected chicken were similar in both infected-infectious (Mem Live) and infected-non-infectious (Mem Noninf) blood. We also found that the negative effects of infection and stress on EBS were not restricted to the first oviposition cycle, but rather that these factors could lead to a dramatic decline in the lifelong reproductive success of individuals. Our results highlight both the life-long and indirect (i.e. due to anemic blood) fitness costs of Plasmodium infection to both stressed and unstressed mosquitoes. Such costs are important from an ecological and epidemiological perspective, as they could affect evolution of resistance/tolerance mechanisms, and in turn affect mosquito population dynamics and vector potential.

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MOSQUITO COMMUNITIES AND VECTOR-ASSOCIATED MICROBIOMES SAMPLED ACROSS A HABITAT GRADIENT OF THAILAND

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Changes in biodiversity have the potential to affect the risk of infectious diseases in plants and animals, including humans, since infectious disease distribution is largely dependent inter-specific interactions. In particular, mosquito-borne diseases are well-suited to study how changes in interacting species, namely mosquitoes, their hosts, and associated microorganisms in changing habitats may affect infectious disease risk. Current knowledge of mosquitoes and their associated microbial communities in natural habitats is, however, limited. Here we explored the composition and diversity of mosquitoes and mosquito-associated microbes in relation to habitats ranging from forest to urban areas in the central plain of Nakhon Nayok province, Thailand. During the rainy season in 2008, adult mosquito collections from 24 sites using CDC light traps, BG sentinel traps, Mosquito Magnet traps, and CDC backpack aspirators yielded a total of 62,511 identifiable female mosquitoes of 54 confirmed taxa. Female mosquito abundance was highest in the rice field habitat and lowest in the forest habitat with 27,041 (43.26%) and 4,840 (7.74%) mosquitoes collected, respectively. The diversity of mosquito communities was characterized using a variety of diversity measurements including statistical sampling approaches to extrapolate species richness. In general, the rural habitat was the most diverse while the least diverse habitat varied depending on the indices used. The Vishnui subgroup of Culex species was the most common taxon found overall and also the most common in the fragmented forest, rice field, rural, and suburban habitats, while Uranotaenia sp. was the most common taxon in the forest habitat and Cx. quinquefasciatus was the most common species in urban settings. Aedes aegypti and Ae. albopictus were most abundant in urban and rural area respectively. To explore the diversity and composition of vector-associated microbiomes, the microbiota from three vector species Cx. quinquefasciatus, Ae. aegypti, and Ae. albopictus from different habitat types were studied using 454 pyrosequencing of ribosomal RNA. Patterns of microbiota community assembly in mosquitoes by habitat type and vector species using both alpha- and beta-diversity analyses will be discussed. Our results are particularly relevant for understanding the dynamics of mosquito vectors and their associated microbiomes in landscapes of Thailand.

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LINKING OVIPOSITION-SITE CHOICE TO OFFSPRING FITNESS IN Aedes aegypti: CONSEQUENCES FOR TARGETED LARVAL CONTROL OF DENGUE VECTORS

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Maternal oviposition-site choice and its repercussions for offspring fitness are known to influence population dynamics of insects. Using four experimental container treatments (size [large vs. small] x water management [manually filled vs. unmanaged]), we tested the hypothesis that wild Aedes aegypti in Iquitos, Peru choose egg-laying sites to maximize offspring survival and growth. Among 80 containers located
in 20 houses, females consistently laid more eggs in large vs. small containers ($\beta = 9.17$, $p < 0.001$), and in unmanaged vs. manually filled containers ($\beta = 5.33$, $p < 0.001$). There was poor correlation, however, between oviposition preference and two components of mosquito fitness, puation probability and adult size. Probability of puation was higher for mosquitos developing in small, unmanaged containers than any other container type ($\beta = 3.4$, $p < 0.001$). Adult body size decreased for individuals developing in large containers (females: $\beta = -0.19$, $p < 0.001$; males: $\beta = -0.11$, $p = 0.002$) and unmanaged containers (females: $\beta = -0.17$, $p < 0.001$; males: $\beta = -0.11$, $p < 0.001$). Our data suggest that the majority of Aedes aegypti eggs are laid in non-optimal sites, such that selective oviposition behavior contributes to population regulation by limiting the production and size of adults. Targeted larval control strategies removing the most productive containers may have the unintended effect of encouraging females to spread their eggs more evenly among remaining containers. By tracking egg-laying patterns of individual females inside a semi-field enclosure, we found that the probability of any container receiving eggs increased when preferred container were removed (but the total number of containers remained constant) ($\beta = 1.36$, $p < 0.001$). We suspect that in Iquitos, and possibly other locations, selective oviposition behavior by Aedes aegypti, along with a potential switch from clustering eggs to spreading them out, will render targeted larval control less effective than anticipated.

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TOWARDS A CONSERVED CIS-REGULATORY MODULE WITH CROSS-STRAIN/SPECIES APPLICATION FOR DRIVING ANTI-PATHOGEN EFFECTOR TRANSGENES: COMPARATIVE TRANSCRIPTOMICS TO DISCOVER EARLY BLOODMEAL-RESPONSIVE, CIS-REGULATORY SEQUENCES FROM MOSQUITO MIDGUT RNA-SEQ
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Empirical definition of active cis-regulatory elements (CRE) through classical “promoter bashing” is difficult in mosquitoes due to the time and effort required to produce transgenic mosquito strains. Bioinformatic methods combined with existing biological knowledge and quality mRNA abundance data should allow the inference of active CRE combinations, cis-regulatory modules (CRM), without requiring construction of transgenic mosquito strains. The ecdysone (20E) response cascade is conserved throughout insects and has been shown to drive changes in mRNA abundance following the ingestion of a bloodmeal. This supports the hypothesis that it should be possible to deduce a conserved CRM by studying bloodmeal-regulated transcript abundance across multiple mosquito species. 20E has had multiple early-response factors described previously including the ecdysone receptor (EcR), its binding partner ultraspireacle (USP), and the 20E-inducible gene E74. Other laboratories have shown that levels of 20E early-response factor isoforms vary in a time- and tissue-specific fashion in response to pulses of 20E following a bloodmeal. This allows one hormone to regulate diverse cellular responses.

Tissue-specific, time-course RNA-Seq data with high temporal resolution (2 hours) will be used to compare 20E early-response factor isoform mRNA expression levels across evolutionarily distant species (Anopheles gambiae, Aedes aegypti, and Culex quinquefasciatus) to infer transcripts that display probable time-lagged induction by 20E and harbor known 20E early-response factor motifs. This transcript set will leverage a combined comparative-genomics and expression-profile based CRE/CRM discovery strategy to reveal putative CRMs expected to provide a better understanding of 20E-regulated transcript regulation in the midgut. The discovered CRMs will serve as the basis for validation of a set of conserved CREs that may be combined to drive robust anti-dengue effector transcription in the midguts of Aedes aegypti mosquitoes directly following the ingestion of each bloodmeal.

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USING MOSQUITO SURVEILLANCE DATA TO PREDICT HUMAN WEST NILE VIRUS TRANSMISSION RISK
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West Nile virus (WNV) has become endemically established across the Americas with enzootic activity and significant human illness. Despite this, funds for surveillance and control are limited and decreasing. Predicting the risk of human infection to initiate timely preventative measures is the primary goal of public health mosquito surveillance. Many arbovirus response plans outlines recommended public health and mosquito control actions based on levels of virus activity determined by statewide mosquito surveillance. However, studies linking mosquito surveillance data to the spatio-temporal risk of human WNV infection, have rarely been attempted. We quantified the links between mosquito surveillance data and the spatio-temporal patterns of 3,827 human WNV cases reported in Colorado from 2003-2007. Mosquito data were strongly predictive for spatio-temporal variation in human WNV infections several weeks in advance in a statewide analysis, and with temporal variation within a county. Correlative and predictive relationships were strengthened by using pooled estimates of prevalence from across the state in estimating risk early and late in the season when few mosquitoes were trapped at the local scale. However, we found that when current year prevalence data was not available, as could occur with reduced or eliminated surveillance budgets, no meaningful predictions of human risk could be made to determine appropriate public health response. Overall, our results demonstrate that mosquito surveillance provides valuable predictive data about the risk of human infection which can be used to trigger emergency response action and allocate limited public health and mosquito control resources.

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DOCUMENTING THE POTENTIAL INTRODUCTION OF DENGUE VIRUS INTO KEY WEST, FLORIDA THROUGH AIRLINE AND CRUISE SHIP PASSENGERS FROM DENGUE-ENDEMIC LOCATIONS
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For the first time in decades, sporadic cases of locally-acquired dengue were reported in Key West in 2009 and again 2010. Current hypotheses regarding this continuance include vertical transmission, the establishment of an endemic state with undetected transmission between years, and multiple introductions via visitors from endemic countries during both years. Regarding the third hypothesis, country- and year-specific dengue incidence data (PAHO) and the numbers of airline passengers originating in dengue-endemic countries in this hemisphere with a final destination of Key West were used to estimate the relative magnitude of potentially viremic travel passengers per year. These estimates suggest multiple introductions per year are not uncommon and that potential introductions in 2009 and 2010 were higher than in 2007 and 2008 as a result of an increase in air travel and major dengue activity in the Caribbean and Central America. Both years were El Niño years that historically are associated with elevated temperatures and higher dengue activity in the region. A similar analysis of potential introductions via the cruise ship industry will also be presented.
CRYPTIC BREEDING: A POTENTIAL CAUSE OF LOCAL DENGUE TRANSMISSION IN KEY WEST, FLORIDA

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June 2009 marked the beginning of a 2-year outbreak of locally-acquired dengue in Key West, Florida. Despite increased control efforts by mosquito control and local residents, the number of dengue cases in 2010 nearly doubled that of 2009. Surveillance on the abundance of immature mosquitoes was inconsistent with magnitude of the adult population of Aedes aegypti. Similar disconnects(?) between immature and adult abundance in other dengue-endemic regions have been the result of cryptic breeding which occurs when mosquitoes reproduce in locations that escape control efforts. The majority of homes in Key West were built prior to municipal utilities and stored water in cisterns and disposed of waste through septic systems. Cisterns and unused septic tanks are several cubic meter in size and most are not easily accessible. Although historical maps exist, the true number of cisterns and septic tanks is unknown thus complicating control efforts. Presented here are the combined efforts of the University of Florida and Monroe County Mosquito Control to identify and eliminate cryptic breeding sources for Ae. aegypti in Key West.

THE QUALITY OF DRINKING WATER IN COMMUNITIES ALONG THE MARANON RIVER IN THE PERUVIAN AMAZON

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Water is one of the world’s most critical resources, however international water quality surveillance and monitoring is often not implemented, obscuring associations and etiologies of potentially related illnesses. We conducted an evidence-based approach to understand the sources and types of water contaminants as well as the overall safety of available drinking water in Peru. A comprehensive, portable, water quality assessment toolbox was used to quantify key microbial (total coliforms, E.coli and enterococci) and chemical (metals, anions and pesticides) contaminants. This assessment system was applied in the field to evaluate the drinking water of 20 rural villages bordering the Maranon River in the Peruvian Amazon. In total, 32 households, 32 drinking water sources, and 2 water treatment systems were assessed. All household drinking water samples and 93% of source water samples contained moderate to high levels of E.coli contamination. Water treatment systems varied in contaminant removal, ranging from 2.03 logs to 4.15 logs of measured bacterial removal. Multiple water samples contained chemical contaminants in excess of WHO guideline levels including phosphate (anion); aluminum, iron, and manganese (metals); and lindane (pesticide). Current international water quality screening and evaluation efforts are not adequate to address the burdens caused by the adverse health effects of waterborne contaminants, thereby demonstrating the need for portable water quality screening. In the Peruvian Amazon, results comparing source water and household contamination suggest recontamination during transport. Analysis of chemical pollutants revealed a need for water treatment to address metal contaminants. Treatment system results indicated that standardized treatment measures are required. The use of our water quality assessment toolbox provided more comprehensive detection and analysis of waterborne threats to the public. These data can help local governments and non-governmental organizations to select appropriate treatment solutions.

INTEGRATION OF A SAFE WATER SYSTEM WITH ANTENATAL SERVICES, MACHINGA DISTRICT, MALAWI, 2010-2011

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Antenatal clinic (ANC) visits provide an opportunity to integrate additional interventions to improve maternal and neonatal health and motivate pregnant women to attend ANC services. In Malawi, although 93% of women attend at least one ANC visit, 57% deliver in health facilities, and 7% have postnatal checks. To reduce the risk of diarrhea, a leading cause of childhood mortality, we integrated free hygiene kits (safe water storage containers, water treatment solution [WaterGuard], soap, and oral rehydration salts) with ANC services. To receive the hygiene kit, women had to have a spouse/partner present; HIV testing was also offered to the couple. At subsequent ANC visits, up to 3 refills of WaterGuard and soap were provided. We surveyed 106 women receiving ANC care at baseline before program implementation and at follow-up 12 months later to assess water treatment; test drinking water for residual chlorine; observe hand-washing; and determine ANC service utilization. From baseline to follow-up, there was an increase in the percentage of women who had ever used WaterGuard (38% vs. 100%, p<0.001), knew how to use it correctly (23% vs. 81%, p<0.001), were observed to have a bottle in their home (3% vs. 77%, p<0.001), had residual chlorine in their stored water (0 vs. 71%, p<0.001), and were able to demonstrate proper handwashing technique (21% vs. 65% p<0.001). At follow-up, 89% of respondents had ≥3 ANC visits, 90% delivered at a health facility, 99% were tested for HIV, 99% of partners were tested for HIV, and 98% had disclosed their status to their partner. Women in this program showed statistically significant increases in water treatment and hygiene practices, and high utilization of ANC services and HIV testing. This evaluation suggests that integration of hygiene kits, refills, and HIV testing during ANC is feasible, can serve as an incentive to increase use of health services, and may help motivate changes in health behavior.

IMPACT OF COMPLEXITY OF HANDWASHING INSTRUCTIONS ON ADHERENCE IN A LOW INCOME SETTING, DHAKA, BANGLADESH, 2010

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Handwashing reduces diarrhea risk in young children. Interventions to improve handwashing usually include instructions on how and when to wash hands. These instructions vary in complexity, with some recommending multiple steps including duration of lathering and scrubbing various aspects of the hands. To assess whether complex handwashing instructions result in reduced adherence, we conducted a randomized trial in a low-income area of Dhaka, Bangladesh. Mothers of young children were randomly assigned to one of three sets of handwashing instructions: simple, moderate, or complex. Simple instructions were to wet, lather, and rinse hands; moderate instructions included simple instructions and additional steps to scrub palms, scrub backs, and dry hands by waving them in the air; complex instructions included moderate instructions and additional steps to scrub between fingers, scrub under nails, and lather for 20 seconds. The field worker...
taught the participant the randomly assigned set of instructions, without mention of the other two sets. Immediately, two days, and two weeks after the teaching, participants were asked to demonstrate handwashing to the field worker. Adherence was defined as demonstration of all of the instruction steps prescribed for the assigned treatment arm. We enrolled 244 participants (simple n=85, moderate n=75, complex n=84). Compared with the simple group, in which 100% adhered to prescribed instructions at all post-intervention assessments, the more complex groups had lower adherence at two weeks (moderate 42%, p<.0001; complex 31%, p<.0001). Adherence to air-drying hands was low at immediate, Day 2 and Week 2 assessments (moderate: 49%, 39%, and 47%; complex: 57%, 46%, and 38%). Exclusion of the air drying step from the outcome yielded adherence rates of 99%, 91% and 88% for the moderate group and 81%, 69% and 71% for the complex group. In a low-income community in Dhaka, highly complex instructions for handwashing resulted in decreased adherence. Future research should investigate whether adherence to the highly complex set results in greater hand decontamination than adherence to the simple or moderate set of instructions. When developing materials to promote handwashing behavior, handwashing promotion programs should consider the complexity of the overall set of instructions, as well as the microbiological impact and feasibility of adherence to specific instructions, such as air drying.

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CONSISTENT SOAP AVAILABILITY CORRELATES WITH USE OF INEXPENSIVE SOAP PRODUCTS AND IMPROVED HANDWASHING BEHAVIOR IN LOW-INCOME HOUSEHOLDs IN DHAKa, BANGLADESh

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Handwashing (HW) with soap reduces diarrhea in children < 5 years of age in low-income countries. Understanding characteristics of households with increased HW could inform interventions to increase this behavior. Amongst low-income households in Dhaka, Bangladesh, we studied consistent soap availability as a possible indicator of increased HW. Households were selected randomly from controls of a case-control study on hygiene and respiratory illness; all had ≥ 1 child ≤ 5 yrs. We visited 220 households 8 times over 4 weeks in Feb-Mar 2010. Respondents were interviewed about soap availability. Fieldworkers observed the presence of soap and water at HW stations, the cleanliness of respondents’ palms and administered a validated 14-question tool on the strength of handwashing habits. We used data from structured observations, conducted several months prior, to estimate HW behavior at critical times. We used logistic regression to adjust for socioeconomic status, and compared households that had soap for HW available at 100% of visits to households that did not. Soap for HW was available in 1513 (88%) of 1716 visits to 220 households. In 110 households (50%), soap was available at every visit. Compared to those with inconsistent soap availability, households with consistent soap availability at each visit were more likely to be in the highest socio-economic status quintile (determined by principal component analysis on household assets) (OR 1.9; 95% CI 1.4—2.4), more likely to have soap present at the HW station (OR1.6; 95% CI 1.1—2.0), more likely to wash hands with soap at critical times (OR 1.4; 95% CI 1.1—1.7) and more likely to identify cheaper detergent soap rather than bar soap as the main HW product (OR 2.2; 95% CI 1.6—2.9). Consistent availability of soap for handwashing was not associated with scores for the key components of habit. Households that had soap available in the home during each visit were also more likely to keep soap where it was needed for washing hands, and to wash hands more frequently at times relevant for hand contamination and pathogen transmission. Reliance on less expensive soap may facilitate consistent soap availability, underscoring the importance of promoting affordable means of increasing handwashing. Interventions that emphasize soap availability and the efficacy of inexpensive soap may be effective in increasing handwashing behavior at critical times.

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IMPACT OF INTENSIVE HANDWASHING PROMOTION ON HOUSEHOLD TRANSMISSION OF INFLUENZA IN A LOW INCOME SETTING: PRELIMINARY RESULTS OF A RANDOMIZED CONTROLLED CLINICAL TRIAL

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Although handwashing with soap decreases the risk of all-cause respiratory illness, there is little published empirical evidence for the efficacy of handwashing with soap for prevention of influenza transmission in resource-poor settings. We tested the impact of handwashing promotion on the risk of household transmission of influenza, influenza-like illness (ILI), and fever in rural Bangladesh. In 2009 and 2010, we identified index case patients (ICPs), individuals who developed ILI within the previous two days and were the only symptomatic person in their household. ILI was defined as fever in children <5 years old and fever with cough or sore throat in persons > 5 years old. Households were randomized to intervention or control). The intervention group received handwashing stations with soap and daily handwashing motivation at critical times for pathogen transmission, such as after coughing or sneezing. We conducted daily surveillance and tested household members with fever for influenza viruses by polymerase chain reaction. Secondary attack ratios (SAR) were calculated for influenza, ILI, and fever in each arm. We used logistic regression with generalized estimating equations to estimate the significance of the SAR comparison while controlling for clustering by household. Among 274 ICPs enrolled, 33 (12%) had laboratory-confirmed influenza infections. The SARs for influenza among household contacts of ICPs with confirmed influenza virus infection were 7.5% in the control arm (10/133) and 11.0% in the intervention arm (11/100) (p = 0.362). The SAR for ILI among household contacts of all ICPs was 11.9% in the control arm (146/1,226) and 14.2% in the intervention arm (168/1,314) (p = 0.232). SARs for fever were 12.1% and 15.0%, respectively, in the control and intervention groups (p = 0.113). When an intensive handwashing intervention was initiated after illness onset in a household member, we found no protective effect against influenza virus infections. Handwashing behavior may not have changed rapidly enough to match the pace of influenza virus transmission between household members. Courtesy bias among intervention households, who received daily motivation as well as hardware to facilitate handwashing, may have led to greater reporting of respiratory symptoms. Future efforts should consider whether handwashing behavior can be changed quickly after illness onset in order to blunt household influenza transmission.

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CONSUMER INPUT TO DESIGN AND DEVELOPMENT OF A NOVEL HOUSEHOLD WATER TREATMENT DEVICE

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We collected consumer preference data in urban, periurban, and rural areas in India and Indonesia to use in design and development of a novel POU device for use in Asia. The end product incorporates a

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drinking water disinfection medium (registered by USEPA-#72083-3, 2009). Consumer exposures ranged from 1-month in-home use of functional prototypes, to use-pattern questionnaires, and from household placement of life-size cut-outs of proposed designs to 3-dimensional models based on these designs. Householders showed a preference for gravity feed device configurations that: could accommodate ~ 10 L of source water; allow for collection of filtered, disinfected product water after no more than a few hours; ensured collection of clear, uncolored water with no detectable taste, taint, untoward mouth-feel or odor on immediate consumption or after storage; offered ease of use in cleaning of upper chamber filtration elements; ensured high convenience in secure replacement of the water treatment train (prefilter/filter/adsorption media/disinfecting cartridge) after a useful life of no less than several months’ daily use (i.e., > 1000L); required minimal assembly at start-up; provided for ready access to product water via a faucet/outlet with reliable, drip-free function; and (critically important) had a ‘modern’ and attractive appearance, enhancing the household working and living environment. From in-home observations we determined that: construction needed to be robust, include auto-shut-off at the end-of-life, plus a visual indicator of approaching termination, and include an option for ‘dialing in’ varying efficacy levels (up to US-EPA 6/4/3). HaloPure Waterbird emerged from this process, a gravity-feed purifier capable of 6/4/3 log reduction, auto shut-off at 1500L (~20%), and leak-free cam-lock cartridge placement. Imperceptible halogen residual provides for continued protection of product water, in the device or upon transfer. Listening to the “voice of the consumer” can lead to enhanced product design aimed at household water treatment device development.

USE AND ACCEPTABILITY OF A POINT-OF-USE WATER FILTRATION DEVICE IN HIV-1 INFECTED ART NAÏVE KENYAN ADULTS

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Among HIV-infected adults and children in Africa, diarrheal disease remains a major cause of morbidity and mortality. WHO recommendations suggest HIV-infected individuals should treat drinking water at the point-of-use. While simple and effective water filtration devices are available, limited data exist regarding the use and acceptance of these devices in this population. We enrolled ART naïve HIV-positive adults into a two-year cohort study in western Kenya. Individuals were visited in their home at least once to assess acceptability and use of a study water filtration device. Of 417 participants enrolled and subsequently visited, most were female (81%), married (64%), had at least a primary school education (72%), and had CD4 cell counts above 350 cells/µl (76%). At enrollment, participants reported the most common sources of drinking water to be shared pipe or tank source (45%) followed by well water (25%) and river or stream (25%). Among participants with a functioning device, more than half (57%) reported using the water filtration device in all of the last 5 instances of obtaining water to drink (always) and 25% reported using the device at least 3 of the last 5 times. Only 3% reported never using the device. We found household monthly income greater than 5,000 Kenyan Shillings (~$57 US) to be associated with always using the device (OR: 2.12 [95%CI 1.23, 3.65]). A trend towards an association between increasing age and always using the device was also observed (OR per 5 year increase: 1.11 [95%CI 0.98, 1.30]). While 38% of participants reported drinking water outside of the home within the last 24 hours, most (77%) reported filtering their drinking water. Almost all participants found the device very acceptable, with 97% willing to purchase the water filtration device if their current device were to break. Providing simple point-of-use water filtration devices to HIV infected adults may be an inexpensive and practical intervention to improve water quality and reduce the risk of diarrheal disease among this high-risk population.

EFFICACY VERSUS EFFECTIVENESS OF WATER CHLORINATION IN RURAL COASTAL ECUADOR

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Chlorination can provide a low-cost method of treating drinking waters and is known to be efficacious for reducing bacterial loads, but actual effectiveness under household conditions may not reduce microbial contamination to the same extent as under lab conditions. In a previous study we found no significant differences in log reductions in drinking water of households that reported chlorination of their water in rural coastal Ecuador. We present the results of a follow-up study at the same field site in which we observed and quantified chlorination procedures at the household instead of relying on self-reported chlorination. We also tested source waters and water from control containers stored under protected conditions outside of the household. We collected three sets of samples (source water, water stored in the home, and water stored under control conditions) from 145 households: 67 that did not chlorinate, 42 that used locally available chlorine according to local practices, and 35 that used chlorine dosed to recommended standards. Covariates included physicochemical data and household level indicators. The efficacy of chlorine treatment in our field laboratory-matched control samples was higher than the effectiveness in corresponding household samples, which is most likely the result of recontamination in the household during storage. Recontamination of water in containers in the household over a 24-hour storage period was observed between pairs of household and matched control samples for both E. coli and total coliform concentrations, with mean log differences ranging from 0.4017-0.6147 (p < 0.0001). 63.8% of samples had greater microbial contamination in household samples than in their matched control. The reduced effectiveness can also be explained by other factors such as source water turbidity, socioeconomic status, unsafe water storage behaviors. Negligible disparities were found between the two chlorine treatment groups, suggesting that dosing practices did not greatly modify the relationship between chlorination and log reduction in contamination. Household effectiveness of chlorine treatment was significantly reduced over laboratory efficacy. This research provides important new insight about the relationship between household storage practices and chlorination under village conditions.

ROTAVIRUS OUTBREAK AMONG CHILDREN IN DAY CARE CENTER, ZAPORIZHZHYA, UKRAINE

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In the city of Zaporizhzhya the incidence of acute gastroenterocolitis (GI) has recently increased. The proportion GI illness due to rotavirus infection (RI) has increased from 21.6% in 2008 to 40.6% in 2009. Among children incidence of RI has increased 2.5 times. We investigated an outbreak of GI in a daycare center (DCC). Samples of drinking water, food and human specimens were examined bacteriologically for intestinal pathogens. Ill persons, contacts and water were tested for rotavirus antigen by ELISA and dipstick testing. During a two week period in April, 17 cases of RI were reported. Cases were identified in 8 of 11 classes. In a class which attended only three hours per day there were no cases. Through testing we identified 11 carriers (1 caregiver from class 1 and 10 children). The highest incidence of RI (4 patients and 7 carriers) was observed in class 1 which consists of children under 3 years. The primary case was identified in this class. Rotavirus was found in these 4 children. Due to a staffing shortage care-givers served food to the children against normal sanitary
regulations. Using a retrospective cohort study design we established that the route of transmission was beet salad (RR=3.5; CI 1.07-11.36). The salad was served from a single bowl and distributed to children by class. Children from the two classes in which there were no cases of RI received the salad first. The first 4 cases of RI were not identified until laboratory testing was performed. The study established that the caregiver was infected at DCC. Transmission is believed to have occurred via asymptomatic carriers. Caregivers serving food to the other classes are thought to have transmitted disease to them. No cases of RI occurred in classes that received salad before class 1 was served. This study demonstrates the necessity of strict adherence to the sanitary and hygiene regulation in DCC’s and the ongoing problem of RI.

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SPATIO-TEMPORAL PATTERNS OF DIARRHEAL DISEASE CAN REVEAL TRANSMISSION PATHWAYS IN AN EMERGING URBAN REGION OF ECUADOR

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Diarrheal disease is caused by a variety of pathogens that exploit multiple transmission pathways. The patterns of diarrheal disease in space and time may reveal which transmission pathways are dominant; e.g., direct person-to-person spread produces temporary clusters of cases, whereas environmental pathways result in constant clusters around environmental sources. We explored these spatial and temporal distributions of diarrhea in Borbón, a small urban region of northwestern Ecuador. The relationship between these patterns and household and neighborhood WASH characteristics was also estimated. We conducted a series of six nested case control studies between December 2008 and May 2009. Surveys were carried out monthly to collect data on WASH factors. The river as well as all houses and outdoor latrines were mapped using GPS. We employed spatial point pattern analyses assuming an inhomogeneous Poisson process. We used the K-function to measure clustering and the ratio of intensity between cases and controls to estimate spatial variation of risk by month. Generalized linear and generalized additive models were used to estimate the association between WASH factors and household diarrhea. We found both spatial and temporal variation of diarrhea in Borbón. The spatial variation was associated with different risk factors each month; the exception to this finding was living with children under five, which was found to be a consistent risk factor. For example, early in the rainy season (December and January), use of an unimproved sanitation facility was significantly associated with diarrhea. In the middle of the season, significant WASH effects were absent. Towards the end of the rainy season (May) better household hygiene was significantly protective for diarrhea. These results provide insight on where and when improvements to WASH factors may protect from diarrheal disease, highlighting the importance of indirect transmission through contaminated latrines in the dry season.

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RAPID VIABLE DETECTION OF HUMAN-ORIGINATED FECAL CONTAMINATION USING IMS/ATP AND qPCR TARGETING BACTEROIDES FRAGILIS

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Human-originated fecal contamination of our drinking water source and recreational water is a continuous public health concern around the world. Timely and cost-effective ways in detecting contaminants in water is very important for protecting human exposure to possible presence of potential enteric infectious agents. This study aimed to determine the effectiveness of a rapid detection method, immunomagnetic separation coupled with ATP bioluminescence (IMS/ATP) and qPCR targeting Bacteroides fragilis for human-specific contamination. B. fragilis is a strict anaerobic bacteria and is known to be one of the predominant microbial flora in human gut. For this, an on-site wastewater treatment system was used as a testing ground. Water samples were collected from various points: septic tank effluents; after bioreactor; and after chlorine dioxide treatment. The level of B. fragilis were tested with IMS/ATP using B. fragilis-specific antibody attached magnetic beads and qPCR targeting gyr B gene. The B. fragilis (Bf) levels measured by IMS/ATP showed 1.5 log reductions after bioreactor, and 2.0 reductions after CI0 treatment, respectively, when compared with the original levels in the septic tank. The Bf levels determined by qPCR showed 1.6 log reduction after bioreactor and 2.3 log reduction after CI0 treatment. The Bf levels measured by IMS/ATP and qPCR correlated well (y=0.8834x+0.8791, R = 0.998). In summary, IMS/ATP rapidly determined the levels of Bf in an on-site wastewater treatment system with sensitivity and specificity. Thus, it can provide near real-time (1.5 hr) results of the on-site wastewater treatment efficiency prior to its release into the environment. This is the first study that the new IMS/ATP assay targeting Bf was applied for determining on-site wastewater treatment efficiency. This assay can be applied for a broad range of rapid detection of human-specific fecal contamination in water where fecal contamination is suspected.

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FURTHER INSIGHTS INTO THE PHYSIOLOGICAL MECHANISMS THAT UNDERLIE TSETSE’S BENEFICIAL SYMBIOSES

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Bacterial symbioses are ubiquitous in nature, yet to date few studies have been performed to determine the physiological mechanisms that underlie these relationships. Insects represent a group of advanced multi-cellular organisms that harbor well-documented symbiotic associations. One such insect, the tsetse fly (Glossina spp.), harbors 2 maternally-transmitted bacterial symbionts, mutualistic Wigglesworthia and commensal Sodalis, that are intimately involved in maintaining the overall fitness of their host. In this study we examine the functional mechanisms that underlie these symbioses by producing tsetse flies that lack all of their endogenous microbiota. The resulting aposymbiotic offspring are highly susceptible to infection with normally non-pathogenic E. coli, and this immuno-compromised phenotype is characterized by the absence of phagocytic hemocytes and the irregular expression of immunity-related genes. When hemocytes collected from wild-type tsetse are transplanted into aposymbiotic flies, the recipient individuals regain their refractory phenotype. We also supplement the diet of pregnant aposymbiotic females with Wigglesworthia and Sodalis in an attempt to compliment the fitness of their offspring. Our observations provide further insights into the evolutionary adaptations that anchor the steadfast relationship shared between tsetse and its symbiotic microbes.

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PUNIQUE VIRUS, A NOVEL PHLEBOVIRUS, RELATED TO SANDFLY FEVER NAPLES VIRUS, ISOLATED FROM SANDFLIES COLLECTED IN TUNISIA AND ITS POTENTIAL IMPACT ON PUBLIC HEALTH

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Sand flies 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 for North Africa. Sand flies were collected from the site of Utique, a well-known site of visceral leishmaniasis in northern Tunisia, during the summers of 2008, 2009 and 2010. In 2008 and 2009 sand flies were captured and pooled by sex and species. A vast majority of sand flies belong to Phlebotomus perniciosus. Thus species identification was abandoned in 2010 and sand flies were pooled by sex. Sand flies were tested for the presence of phleboviruses by PCR. Viral RNA corresponding to a novel virus closely related to Sandfly fever Naples virus (SFNV) was detected in pools of sand flies collected in 2008 and 2009. 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 the species. This novel virus was provisionally named Punique virus. Viral sequences in the polymerase gene corresponding to another phlebovirus closely related to but distinct from Sandfly fever Sicilian virus (SFSV) were obtained from positive pools collected in 2008 and 2010. Isolation of this virus temporarily named Utique Virus remained to be determined. The public health impact of those two new viruses remained to be determined.

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FIELD EVALUATION OF A WICKING ASSAY FOR THE RAPID DETECTION OF RIFT VALLEY FEVER VIRAL ANTIGENS IN MOSQUITOES (DIPTERA: CULICIDAE)

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Rift Valley fever virus (RVFV) causes outbreaks of severe disease in domestic ungulates as well as humans in Africa. There is a concern that outbreaks of RVF may continue and that this virus may spread into regions where it had not previously been detected. Surveillance and rapid detection are critical to the initiation of an effective disease control program. Here we report on the field evaluation in Kenya of the VectorTest® RVFV antigen assay, modeled on the VecTest® assay for West Nile virus. The dipsticks provided results in less than 20 min, were easy to use, and did not require a laboratory with containment facilities. Although none of the field-collected mosquitoes were infected with RVFV, the dipstick provided a clear positive result with pools of field-collected mosquitoes spiked with a single positive, irradiated (to inactivate an infectious virus) mosquito. Similarly, the dipstick was able to detect virus from pools of mosquitoes captured during the RVFV outbreak in 2007. The RVFV dipstick assay was highly specific with only a single weak false positive out of 266 pools tested (specificity >99.6%). The RVFV assay can provide a rapid, safe, easy to use preliminary test to alert public health personnel to the presence of RVFV in mosquitoes in a given area. Results from this assay will allow for more rapid medical threat assessments and the focusing of vector control measures on high-risk areas.

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IDENTIFICATION OF A NEW GROUP OF LACTATION-ASSOCIATED PROTEINS IN THE TSETSE FLY, GLOSSINA MORSITANS MORSITANS

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Tsetse females generate a single larva during each gonotrophic cycle. All nutrients for larval development are provided by the mother in the form of lactation products generated by the milk gland. The nutrients within the milk are primarily composed of equal amounts of lipids and proteins. Four proteins have been associated with tsetse lactation products, milk gland proteins 1-3 (gmmmgp1-3) and transferrin. However, little is known about other protein components of tsetse milk. In this study, we performed an Illumina based transcriptome analysis of differential gene expression in pregnant flies compared to those post parturition to identify lactation-specific genes. This analysis revealed 11 transcripts that are upregulated during pregnancy including the previously identified gmmmgp1-3 and transferrin. Seven new MGPs (gmmmgp 4-10) were identified in this analysis. These proteins appear to be related as the amino acid composition of these proteins is similar to gmmmgp2-3. Genomic analysis of gmmmgp2-10 revealed that they are located on the same genomic loci. Analysis of the predicted upstream regulatory regions for gmmmgp4-10 found conserved binding sites previously identified in the regulatory regions for gmmmgp1-3. Search for homologues sequences to these genes has only revealed a single uncharacterized sequence from the flesh fly, Sarcophaga crassipalpis. The predicted amino acid sequences for gmmmgp2-10 contain a high percentage of hydrophobic amino acids and a conserved secretory signal peptide, however they lack characterized functional domains. Expression patterns of gmmmgp2-10 are female specific and localized to the milk gland tissue. Temporal analysis of transcript levels for these genes is similar to the other genes...
associated with lactation. This expression pattern results in increased transcript levels in correlation with larvigenesis followed by immediate decline after parturition (birth). Knockdown of gmmmp7 utilizing siRNA injections resulted in a significant reduction of fecundity. The discovery of gmmmp4-10 reveals a family of genes essential for viviparity and novel in function.

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RETENTION OF DUPLICATED LONG WAVELENGTH OPSIN GENES IN THE GENOMES OF THE MOSQUITO VECTORS Aedes aegypti, Anopheles gambiae and Culex quinquefasciatus

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Understanding the role of mosquito vision in mating, host detection and oviposition, may help to improve or develop new control strategies to reduce the incidence of vector-borne diseases. Here we report the first molecular analysis of light receptors (opsins) from three mosquito vectors - the yellow fever mosquito, Aedes aegypti, the malaria mosquito, Anopheles gambiae, and the southern house mosquito, Culex quinquefasciatus. Opsins are receptors that interact with photons to initiate visual processes. Typically, insects have three classes of opsins that are stimulated by ultraviolet, short, and long (LW) wavelengths. We used expression data to improve the 10 A. aegypti and 11 A. gambiae opsins gene models, and we report the first manual annotation of 13 opsin genes from C. quinquefasciatus. Opsin transcripts were confirmed using published expression data and Reverse Transcriptase-PCR. Phylogenetic analyses predicted six putative LW opsins in A. aegypti, six in A. gambiae and eight in C. quinquefasciatus, suggesting an expansion of these genes in the Culicidae relative to other insect taxa. Time of divergence suggests the mosquito LW opsins originated from several duplication events between 167 to 1 million years ago (MYA), and that 15 LW genes may have originated following a duplication event that occurred approximately 126 MYA. LW opsins share approximately 100% and 60% amino acid similarity within and between mosquito taxa, which raises intriguing questions regarding the retention of these genes in the three mosquito genomes. Seven amino acids were identified under positive selection in the N and C termini, and one in a third trans-membrane domain suggestive of opsin spectral tuning. Conserved nucleotide sequence in 6 out of 38 ortholog pairs and in 8 out of 14 paralog pairs of the non coding regions, up- and or down-stream, of the LW opsins is indicative of coordinated gene regulation. We discuss potential mechanisms, including positive selection and differential gene regulation, for the conservation of LW opsins in these mosquitoes.

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FIELD USER ACCEPTABILITY EVALUATION OF A NOVEL, SELF-SUPPORTING, LONG-LASTING INSECTICIDAL NET (LLIN)

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Insect bed nets provide protection against arthropod-borne disease pathogens such as malaria, dengue, and leishmaniasis. United States Army service members currently have a choice between two types of bed nets to use in field environments; however, both have various limitations that preclude effective long-term use by non-mobile forces. Therefore, the US Army was faced with a challenge to develop an improved bed net that does not have any of the limitations associated with these existing bed nets. The Walter Reed Army Institute of Research has partnered with Tritons Systems, Inc. to develop a novel, self-supporting, long-lasting, insecticide-impregnated net (LLIN). The purpose of this study was to evaluate the new bed net in comparison with the existing Standard and Self-Supporting Low-Profile bed nets using an acceptability threshold of 70%. Upon completion of a large scale field training exercise in which these bed nets were used over the course of several nights, soldiers completed a self-administered survey answering questions about their ease of use, setup, dismantling, and comfort. Results of this acceptability study will be presented in the context of military force health protection.

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IMMUNOGENIC AND BIOCHEMICAL PROPERTIES OF IXOLARIS, A TICK SALIVARY TISSUE FACTOR PATHWAY INHIBITOR

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Ixolaris is a potent Tissue Factor Inhibitor from tick saliva. It binds to Factor X(a) and the binary complex Ixolaris/FX(a) interacts with FVIIa/TF thus blocking the coagulation cascade. Ixolaris has been successfully tested as an antithrombotic in rats, and also displays anti-cancer properties in a glioblastoma model. Because Ixolaris displays therapeutic potential, understanding its immunogenic and biochemical properties is of interest. Here we demonstrate that Ixolaris elutes as approximately 18 kDa protein according to gel-filtration chromatography. Light scattering plot and ultracentrifugation experiments also indicate that Ixolaris is a monomeric protein of approximately 18 Kda. Since the predicted mol mass for Ixolaris is 15.5 kDa, the discrepancy is attributed to glycosilation. This contention has been confirmed by a smear observed by SDS-PAGE and mass spectrometry analysis of Ixolaris. Elisa also demonstrate that Ixolaris is non-immunogenic in rabbits and in mice. Taken together, these results provide further support for the potential therapeutic use of Ixolaris in a number of conditions with abnormal expression of Tissue Factor, including thrombosis, cancer, sepsis and malaria.

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MOLECULAR MECHANISMS OF WOLBACHIA-MEDIATED VIRAL INTERFERENCE

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Dengue is one of the most important arboviral diseases currently threatening human populations, with over 50 million cases in tropical and subtropical regions each year. No treatment or vaccine is currently available for dengue fever. Recently, the endosymbiotic bacterium Wolbachia has been proposed to be used as a tool to reduce mosquito vectorial capacity for dengue viruses through population replacement. Our previous studies showed Wolbachia alone can induce resistance to dengue virus in Aedes aegypti, which was associated with a boosted basal immunity in the Wolbachia-infected mosquito. To understand the molecular mechanisms underlying Wolbachia-mediated viral interference, we examined Wolbachia-induced physiological changes in mosquito by comparison of genome-wide transcriptome between Wolbachia-infected and -uninfected Ae. aegypti. Experiments were also conducted to compare full scale physiological responses of the two groups of mosquitoes to dengue virus infection. We found that the toll signal pathway was prominently activated by Wolbachia in response to dengue virus infection. Interestingly, the genes related to redox stress response systems and mitochondria were strictly regulated by the Wolbachia in Ae. aegypti. Further studies were also conducted to investigate how the toll signal pathway was activated by Wolbachia in Ae. Aegypti. As the effector genes of toll signal pathway, the defensins and cecropins genes induced by Wolbachia were confirmed to play roles in control of dengue infection. Our studies provide evidence to support that Wolbachia induces resistance to dengue virus in Ae. aegypti through modulation of host immunity. We discuss the results in relation to develop Wolbachia-based control strategies for population replacement.
THE INFLUENCE OF HABITAT ON THE GENETIC STRUCTURE OF GLOSSINA FUSCIPES FUSCIPES IN UGANDA AND IMPLICATIONS FOR VECTOR CONTROL

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Human and animal forms of African trypanosomiasis represent a burden to the public health and economy of many African countries. For effective trypanosomiasis management, controlling its vector, the tsetse fly (Diptera: Glossinidae), is necessary, but long-term success in vector control efforts requires a better understanding of tsetse dispersal and breeding ecology. We have collected genetic data over several years in Uganda for a major trypanosomiasis vector, Glossina fuscipes fuscipes (G.f.f.). This genetic information coupled with publicly available environmental data (climate, hydrology, land cover) was used to assess habitat selection and dispersal and breeding capacity of tsetse in Uganda. Connection networks between G.f.f. sampling localities were constructed and a modified inverse distance weighting method was used on these networks to interpolate a ‘landscape’ of genetic variation in Uganda. Genetic variation captured in this way was used with environmental data to carry out environmental niche modeling in Maxent v. 3.3.3. The inferred distribution of G.f.f. represents the flow of genetic information on the environmental substrate of Uganda. We used circuit theory methods implemented in the program Circuitscape v. 3.5.4 to model genetic connectivity of the environmental landscape in Uganda and estimate environmental resistance to dispersal between G.f.f. populations. The environmental ‘friction’ estimates were used to explore local genetic structuring of tsetse flies via spatially explicit principal components analysis (sPCA) with the ‘adegenet’ R package. Environmentally explicit modeling of gene flow provides information about the influence of the environment on genetic variation and connectedness. Environmental-genetic inferences about habitat selection and dispersal in tsetse could substantially improve vector control by helping to identify areas to be targeted for control and minimizing the probability of re-infestation from neighboring areas.

THE ROLE OF BIOFILM FORMATION IN COLONIZATION AND TRANSMISSION OF THE COMMENSAL SYMBIONT SODALIS GLOSSINIDII WITHIN THE TSETSE FLY

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Awareness of diversity and abundance of beneficial microbes has greatly increased with the advancement of molecular technologies. Recently, the influence of beneficial microbes in onset or prevention of disease has been shown, indicating an opportunity for harnessing these microbes for control of disease. Sodalis glossinidius is a gram-negative commensal symbiont of the tsetse fly, the sole vector of the African trypanosome. Sodalis is harbored throughout the fly both intra- and extracellularly, primarily in the midgut tissue in close proximity of the trypanosome and is maternally-transmitted to offspring. The proximity and the ability to genetically manipulate Sodalis makes it a great candidate for paratransgenesis, i.e., expression of antitrypanosomal compounds in the Sodalis within tsetse's midgut. One essential aspect of paratransgenesis is understanding transmission biology of Sodalis and recolonization efficiency of genetically modified Sodalis in tsetse lines. Biofilms are dense populations of microbes that adhere to surfaces and each other secreting extracellular polymers. Only a few studies have shown the role of biofilm formation in vector born disease; i.e., Yersinia pestis within the flea gut. The ability of Sodalis to produce a biofilm was investigated using a classical microtiter plate biofilm assay and was shown to produce a biofilm under in vitro conditions. In this study we assessed genes important for biofilm formation in the fly gut.
colonization process, transmission to progeny and trypanosome infection rates. Our studies provide enhancement of paratransgenic methodology by understanding the role of biofilm formation in both recolonization of Sodalis and trypanosome infections, which will guide us in applying paratransgenesis in the future.

NEW VECTOR CONTROL MATERIALS FROM THE ARMED FORCES PEST MANAGEMENT BOARD

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The Deployed War-Fighter Protection research program (DWP) is an initiative to develop and validate novel methods to protect United States military deployed abroad from threats posed by disease-carrying insects. Starting in 2004 and administered by the Armed Forces Pest Management Board the program is funded at $5M per year. The DWP research portfolio is concentrated in 3 specific areas: novel insecticide chemistries/formulations, application technology, and personal protective systems. Program consists of a non-competitive funding process for USDA ARS-based research, and a competitive grants process open to non-USDA ARS scientists (PIs from academia, industry, and military entomologists: 55 projects funded). Up to $2 million per year is given to USDA ARS National Program 104, dealing with Veterinary, Medical, and Urban Entomology. Ultimate objective is to find industry partners and get useful products into the market/military stock system. Presentation highlights DWP products with examples of equipment, insecticides, and ~300 refereed publications.

COMPREHENSIVE EPIDEMIOLOGICAL RESEARCH EFFORT ON FEBRILE ILLNESSES AND HEMOGLOBINOPATHIES ALONG THE BANGLADESH-MYANMAR BORDER

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In order to estimate the burden of febrile illnesses in the border region of Bangladesh toward Myanmar a comprehensive prevalence study on febrile illnesses and hemoglobinopathies was conducted in an area with suspected high prevalence of malaria and other infectious diseases. Little is known about the prevalence of febrile illnesses in the Chittagong Hill Tracts, the southernmost region of Bangladesh bordering Myanmar and India, an area with limited access to medical care due to inaccessible terrain and lack of infrastructure. Samples were collected from patients enrolled during two separate cross-sectional studies in the years 2007 to 2010 covering the same rural communities in rainy and dry season to assess seasonal trends. In a parallel ongoing hospital-based fever survey data of febrile participants from the catchment area of the Bandarban Sadar Hospital were collected. Out of a total population of 2123 enrolled in the studies 671 acute febrile patients were diagnosed for the most common infectious diseases: malaria (RDT, microscopy and PCR), typhoid fever, leptospirosis, dengue (serological assays) and influenza (RDT and PCR) as well as hemoglobinopathies. The collected data allow for an estimation of long term trends in the epidemiology of the investigated diseases as well as short-term variations such as seasonal fluctuations and emergence of rare conditions. Falciparum malaria remains the major health threat with a cross-sectional prevalence of 40.9% (CI: 35.4 - 46.7%) during monsoon months (May - October). However numbers vary significantly with the season and show an overall declining trend over the years. A high number of seropositive cases for leptospirosis (n=194, 28.9%; SD: 25.6 - 32.5%) and typhoid fever (n=203, 30.3%; SD: 26.9 - 33.8%) indicate a major persistent reservoir of infection for these pathogens in the surveyed communities. Associations of disease distribution with demographic, geographic, and meteorological data were performed to define and map the prevalence and indirect estimates of incidence as the basis for assessing actual disease burden.

INVESTIGATION OF A SUSPECTED OUTBREAK OF ACUTE FEBRILE ILLNESS IN MALINDI, KENYA IN DECEMBER 2010

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Acute febrile illness (AFI) refers to sudden illnesses with fever. It is a common clinical presentation in Kenya where its aetiology remains unknown. Information on the prevalence and causes of AFI in Kenya is limited. Walter Reed Project’s (WRP) AFI surveillance site in Malindi noticed a 3 fold surge in AFI cases from Sept 2010 to Nov 2010 (Sept #10, Oct #5, Nov #15 cases). On average 2 cases are enrolled monthly. This prompted an investigation. Of note cases were from the same locality. Aliquots of malaria negative blood by RDT were sent to WRP reference lab for PCR and ELISA for Malaria, Salmonellosis, Brucellosis, Leptospirosis, Aboriviruses and nasopharyngeal swabs tested for Flu. Study team: WRP and MOH. Study Area: Malindi District Hospital and Kisumu-dogo area. Investigation Period: 15 - 18 Dec. Case size: All 21 cases. A standard questionnaire was administered to all. All lab records from the period were reviewed. Data from the questionnaires was entered into an EPI-INFO database and analyzed. Majority, males (57.1 %, n=12). The median age 30 yr. Most below 20 yr (42.9%, n=9). Most from Kisumundogo (23.8%, n=5). Majority presented with headache (42.9%, n=9), joint aches (28.6%, n=6) and myalgias (19%, n=4). 42.9% of cases classified as having AFI actually had undiagnosed malaria. 42.9% were malaria positive on PCR. All cases were negative for viruses by PCR, Elisas and cell culture. Importantly, the aetiology of fever remained unknown in 57.1% of cases. Malaria RDT’s are not sensitive enough in low malaria transmission areas and when parasitemia is low. A negative RDT may not be enough to rule out malaria in regions of low malaria endemicity. There is clinical and lab evidence of low parasitamia having being cleared in malaria immune patients as no PCR positive was given antimalarials but on repeat PCR all were negative for malaria. This could be explained by self treatment but all denied it. A comprehensive study to discover both common and uncommon pathogen causes of acute febrile illnesses is needed. PCR may be a complement to RDT and Microscopy in low malaria endemic areas. Continue vector control. Malaria naive persons should continue to be offered prophylaxis or preventive measures.

EFFICACY, SAFETY AND PK OF ARTEMETHER-LUMEFANTRINE DISPERSIBLE TABLET IN THE TREATMENT OF ACUTE UNCOMPLICATED PLASMODIUM FALCIPARUM MALARIA IN INFANTS <5 KG BODY WEIGHT

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WHO recommends artemisinin-based combination therapy (ACT) as first-line therapy for infants with uncomplicated Plasmodium falciparum malaria who have body weight (BW) ≥5kg. However, no ACTs are indicated in infants <5kg. Poor safety profile of current standard of care,
EVALUATING THE READINESS OF OUTPATIENT HEALTH FACILITIES TO MANAGE MALARIA CASES IN BENIN

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In 2008, the government of Benin and its partners began implementing a new national malaria case-management policy in 787 public health facilities (HFs) that recommended the use of artesimin-based combination therapy (ACT). We evaluated the readiness of outpatient HFs to manage malaria cases in Benin about 2 years later. In late 2009, we conducted a nationally representative cross-sectional survey of a stratified random sample of 60 HFs. Surveys observed consultations and interviewed and re-examined patients seeking care for any illness and pregnant women seeking antenatal care. In addition, health workers (HWSs) were interviewed, and HFs were assessed to determine the availability of drugs and equipment. Results were weighted. Altogether, 57 HFs, 113 HWSs, and 448 patients were included in the analysis. All HFs had a thermometer, 70.8% (95% confidence interval [CI]: 59.3-82.3%) had a scale for weighing children, and 66.3% (95% CI: 56.6-80.1%) of HFs had a booklet or chart with ACT algorithms. Although all hospitals could perform malaria testing, only 40.8% of non-hospitals could perform testing. In the three months before the survey, 46.7% (14/30) of hospitals and 33.3% (9/27) of health centers had stock-outs of all types of artemether-lumefantrine blister packs (i.e., none in stock) for at least three days. Adherence to the testing policy (i.e., test all patients with a febrile illness, and do not test patients without a febrile illness) was 52.9% (95% CI: 48.3-57.5%) among all 448 patients, 24.7% (95% CI: 18.2-31.2%) among 170 patients <5 years old, and 70.1% (95% CI: 64.7-75.5%) among 278 patients ≥5 years old. Nearly all of the 79 patients who tested positive were given an antimalarial (98.2%; 95% CI: 93.3-100%). However, 22.1% (17/77) of patients who tested negative were given an antimalarial. HF readiness varied. HWSs prescribed antimalarials when tests were positive. However, ACT stock-outs were common, HWSs did not follow testing guidelines for children <5 years old, and they sometimes prescribed antimalarials even when tests were negative.

INVESTIGATION OF A CLUSTER OF DEATHS ATTRIBUTABLE TO MALARIA IN RURAL SENEGAL

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Since 2005, malaria control interventions, including insecticide-treated nets, rapid diagnostic tests, and artesiminin-combination therapies have been scaled up in Senegal, resulting in a large decrease in the malaria-associated morbidity and mortality. However, in Touba district, deaths attributed to malaria from September-November (transmission season) increased by 58% in 2009 compared to 2008, while admissions for malaria decreased 19%. One health center reported 80% (38/47) of the deaths. The National Malaria Control Program led an investigation of these deaths, consisting of interviews with families and care providers and retrospective chart reviews. Charts were reviewed for 38 malaria deaths, all confirmed by rapid diagnostic testing. The median age was 5 years (39% < 5 years and 37% 5-9 years) and 59% were male. Only 17% (6) sought care within 24 hours of symptom onset, with a median of 3 days. Anemia was laboratory-confirmed in 37% (14) and diagnosed clinically in 26% (10); mean hemoglobin was 3.9 g/dL in those tested, two of whom received a blood transfusion. Rapid blood glucose was performed in 18% (7) and complete blood counts in 37% (14). Of the 12 patients with elevated leukocytes, 8 received an antibiotic. Of 37 patients for whom treatment was documented, 65% received a correct medication regimen, 26% had dosing errors, and 5% died prior to starting quinine. The majority (92%) of patients died the day of admission or the following day. Recommendations included expanding the home-based management program, reinforcing preventive community-based interventions, educating the community on danger signs and accessibility of treatment, and staff training to improve referral practices, performance and documentation of history and physical exams, complete blood counts and blood glucose, correct dosing of antimalarials, and differential diagnosis of fever. Additional studies would be needed to determine if the delays in seeking care and deficits in care were associated with the deaths. A standardized tool for investigation of deaths will help improve case management and the response to clusters of deaths.

EFFECTS OF GASTROENTERITIS EPISODES ON MAINTENANCE OF POLIO VACCINE TITERS IN CHILDREN THREE YEARS AND UNDER IN RURAL COASTAL KENYA

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Evidence shows that infants with concurrent gastroenteritis (GE) are less likely to respond to oral polio vaccination than those without gastroenteritis. Our objective was to determine whether further episodes of gastroenteritis in the first three years of life had an effect on maintenance of polio titer. Children enrolled in a birth cohort in rural coastal Kenya received four trivalent polio vaccinations before 6 months of life. Sera were then drawn at 6 month intervals until age 36 months and polio titers were measured using poliovirus IgG EUSA kits. GE episodes were documented during scheduled follow-up visits and at any time of illness during the 3 year period. Student’s t-test was performed to compare those with and without GE at each time point. Of 545 children in the study, 159 had 246 episodes of gastroenteritis in the first three years of life. GE episodes were more likely to occur between 6 and 18 months of life. The range of GE episodes per child was 0-4. Polio titers did not significantly differ between children with and without GE from 6 to 36.
months of age. Although concurrent gastroenteritis may hamper immune response to oral polio vaccine, further episodes of gastroenteritis after the vaccination series do not appear to alter the maintenance of polio titers.

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CLINICAL IMPLICATIONS OF ADHERENCE TO WHO GUIDELINES FOR THE MANAGEMENT OF THE FEBRILE PHASE OF DENGUE

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According to WHO guidelines the use of acetaminophen is indicated during the febrile phase of dengue and aspirin or non-steroidal anti-inflammatory agents (NSAIDs) should be avoided as these drugs may aggravate gastritis or bleeding. However, there is little clinical evidence to support this recommendation. We conducted a prospective cohort study in an endemic area in Colombia to evaluate the potential association between noncompliance with this guideline and the risk of developing severe dengue. Acute febrile outpatients (less than 96 hours of onset) with dengue (confirmed by viral isolation, RT-PCR or a shift from negative to positive IgM test) were followed daily until the seventh day of disease. Subjects were excluded based on the following: diabetes, AIDS, hematologic disorders, cancer or cardiac disease and the presence of a major bleed, albumin < 3g/dl, effusions or shock at presentation. Inappropriate Initial Treatment (IIT) was considered when the patient reported having taken NSAIDs, aspirin or dipyrone. Data collected included signs and symptoms, and daily microhematocrit determinations to recognize hemocconcentration. A complicated case was defined by the following: a platelet count ≤ 100.000/mm³, any spontaneous hemorrhagic manifestation (or one positive tourniquet test); and evidence of plasma leakage (i.e., pleural effusion, ascitis, hypoalbuninemia or a variation of hematocrit greater than 10%). Of 596 patients, 97% appropriately manifested (or one positive tourniquet test); and evidence of plasma leakage (i.e., pleural effusion, ascitis, hypoalbuninemia or a variation of hematocrit greater than 10%). Of 596 patients, 97% appropriately received acetaminophen but 54% also received IIT. 63.2% (n=98) of cases receiving IIT were complicated compared with 36.8% (n=57) complicated cases in the 271 subjects treated only with acetaminophen [OR crude: 1.62; 95% CI: 1.96–6.39; OR: 1.51; IC95%: (1.03-2.2) adjusted by age and sex]. In conclusion, adherence to WHO guidelines during the febrile phase of dengue is important to reduce the risk of complications. This study is registered with Colciencias (Departamento Administrativo de Ciencia, Tecnología e Innovación de Colombia), number: 110245921561.

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MALARIA PREVALENCE AND MORTALITY IN RURAL SIERRA LEONE

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Malaria is a leading cause of morbidity and mortality in rural areas of Sierra Leone. Mortality from malaria is as high as 28% in the under age 5 group of pediatric patients. The Village Medical Project provides medical care and treatment to women and children in several villages in Gorama Chiefdom, Kono District of rural Sierra Leone. The purpose of this study is to document the prevalence of malaria infection, anemia and crude mortality. The project has been working for 3 years to ascertain the success of primary treatment and prevention of malaria in a rural area of Sierra Leone. Adult and Pediatric Patients were tested for Plasmodium falciparum malaria and hemoglobin. Patients were selected from each village based on prior census data and followed for a 2 year period, from 2008-2010. Primary prevention with bed nets were provided for children under 5 years of age. 1043-1463 patients were seen annually over a 2 year period from 2008-2010. Overall, malaria prevalence varied from 67-97%. The overall crude mortality rate from 2008-2010 was 8%. Under age 5 mortality is 9.8%. 87% of the population is anemic based on WHO standards. Access to Medical Care and treatment remains difficult for this population. We are significantly limited by lack of accurate ages, mobility of the population and changing demographics. Malaria is very prevalent in this rural area of Sierra Leone. Primary treatment and prevention has had some impact on mortality rates compared to prior studies, however there still remains a significant disease burden in this area, with significant morbidity and mortality.

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ARTEMETHER, DIHYDROARTESININ AND LUMEFANTRINE DO NOT INDUCE IN VITRO DRUG METABOLIZING ENZYMES AND METABOLISM OF ORAL CONTRACEPTIVES

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The goal of this study was to evaluate in vitro the components of Coartem/Riamet (artemether and lumefantrine) and the active metabolite dihydroartesinin (DHA) for their potential to induce drug-metabolizing CYP enzymes and the metabolism of oral contraceptives. The experiments were conducted according to the FDA drug drug interaction guidance. The assessment was done in vitro in cryopreserved primary human hepatocytes of at least three individual donors. Induction of mRNA, relative to the vehicle control, was determined by real-time PCR and evaluation of changes in cytochrome P450 (CYP) enzyme activities were assessed after 48-h induction periods by LC/MS/MS analysis of CYP-selective probe substrate metabolism. Metabolism of the oral contraceptives was tested by HPLC analysis. Human hepatocytes were incubated with the three test substances up to concentrations which exceeded their therapeutic concentrations by a factor of 10. Ethinyl estradiol and levonorgestrel were selected as active ingredients of oral contraceptives and were tested at their therapeutic concentrations of 1 nM and 20 nM, respectively. Rifampicin at 0.1, 1, and 20 µM, and phenobarbital at 1000 µM were used as positive controls for induction of genes regulated by PXR and/or CAR like CYP2B6, CYP2C, and CYP3A; β-naphthoflavone at 10 µM was included as positive control for AhR-mediated induction of genes like CYP1A. Artemether, DHA, and lumefantrine were determined not to be inducers of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP3C19, or CYP3A enzyme activity in hepatocytes or CYP1A1, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP3A4, or CYP3A5 mRNA. Metabolism of ethinyl estradiol and levonorgestrel was determined not to be induced by artemether, DHA, and lumefantrine. As per FDA criteria, these conclusions were based upon the levels of mRNA or activity at least less than 2-fold, with respect to the vehicle control, and/or less than 40% of the maximal positive control induction response, indicative of a non-inducer in vitro.
Growth Standards 2006. The median number of diarrheal episodes among children in the cohort was 2 (1-3). At 1 yr, 33.9% of infants had chronic malnutrition and 26.9% had acute malnutrition. Three or more episodes of severe diarrhea was significantly associated with chronic (OR=2.45, P=0.02) and acute malnutrition (OR=2.8, P<0.01). Other factors associated with chronic malnutrition were living in a mud house, an indicator of lower socioeconomic status (OR=1.8, P<0.01), presence of an older sibling (OR=1.6, P<0.01). Duration of exclusive breastfeeding, more than primary schooling as highest education in the family and being a girl offered protection of 22% (P<0.01), 44% (P<0.001), 30% (P<0.01) respectively. As expected, severity of diarrhea and poverty are associated with acute and chronic malnutrition, with exclusive breastfeeding and higher education being protective. Lower rates of malnutrition were noted in girls, an unexpected finding.

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U.S. MILITARY FORCE HEALTH PROTECTION POLICIES MAY IMPACT PEDIATRIC MALARIA PROPHYLAXIS PRESCRIBING PATTERNS

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To date, there have been no large scale systematic surveys of antimalarial prescribing practices in the United States. Although pediatric patients are at higher risk of severe disease due to malaria than adults, there is a relative scarcity of information on the prevention of malaria among pediatric travelers versus adult travelers. This study consists of a systematic search of the military health system electronic medical record system for all prescriptions of chloroquine (CQ), mefloquine (MQ), and atovaquone-proguanil (AP) to military family members 8 years of age and under in the years 2005-2010. Prescribing patterns were assessed for changes over time to identify if Department of the Army and Department of Defense policies, published in 2009, limiting the use of mefloquine in deployed forces coincided with changes in prescribing patterns for young children. A total of 3404 prescriptions were written for these medications during the study period. In total, CQ, AP, and MQ, respectively accounted for 7%, 43%, and 50% of all prescriptions. Overall prescription volume increased from a low of 507 prescriptions (60% MQ) in 2005 to a high of 726 (39% MQ) in 2010 (p <0.001). While the total volume of antimalarial prescriptions rose, this change was reflected almost entirely by an increase in the usage of AP. In 2010, in contrast to prior years, 44% of all AP prescriptions were for amounts in excess of a 30 day supply, compared to 37% for earlier prescriptions (p=0.015). This trend of progressively more prescriptions for AP in absolute and relative terms exists over the entire study period (p = 0.003). This study documents that military physicians providing pediatric travel services now prescribe less MQ relative to AP. This occurs even in settings where the duration of travel has led many experts to recommend MQ as the drug of choice. The timing of these changes suggests that military force health protection policy, as well as patient/family and provider awareness regarding adverse effects associated with MQ may be impacting prescribing practices for these medications.

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PRELIMINARY RESULTS OF A HOSPITAL-BASED LABORATORY SURVEILLANCE FOR INFECTIOUS ETIOLOGIES OF UNDIFFERENTIATED FEBRILE ILLNESSES IN GEORGIA

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Since 2008, laboratory-based sentinel surveillance for undifferentiated febrile illness (UIF) has been ongoing in six hospitals to establish the frequency of nine infectious causative agents of febrile illness in Georgia. Hospitalized patients ≥ 4 years of age with fever ≥38°C for ≥48 hours were asked to voluntarily participate. Blood culture and serologic testing (ELISA) were conducted for Leptospirosis spp., Brucella spp., West Nile virus (WNV), Crimean-Congo hemorrhagic fever (CCHF) virus, Coxiella burnetii, tick-borne encephalitis virus (TBEV), hantavirus, Salmonella Typhi and Rickettsia typhi. A total of 478 subjects have been enrolled in the study. Of these, 71% were outpatients and 53% were males with the mean age of 36 years. Fever of unknown origin was the preliminary diagnosis in 88% of patients. Patients also reported having fatigue (90%), rigors (87%), sweating (82%), pain in joints (48%), and sleep disturbances (40%). Acute and convalescent samples from 403 patients (n=473) were initially tested by IgM ELISA. Sixty-nine patients were seropositive for hantavirus (16%), 52 for Leptospirosis spp. (13%), 17 for Coxiella burnetii (4%), 16 for TBEV (4%), and 3 for WNV (0.7%). Additionally, 33 patients were seropositive for Brucella spp. (8%), 3 patients for S. Typhi (0.7%), and 8% (34) of patients showed positivity by IgG ELISA for R. typhi. Highest cross-reactivity was observed for hantavirus and Coxiella burnetii, in 13(2.8%) samples. Preliminary laboratory results indicate a high prevalence of antibodies against hantavirus, leptospirosis, brucellosis and rickettsioses among febrile patients in Georgia. This hospital surveillance for UIF has significantly enhanced laboratory capacity for the detection of specific infectious etiologies as well as established a valuable network of clinical sites that can be used for future syndromic surveillance studies. Confirmed laboratory results will allow the Georgian public health authorities to make better informed decisions regarding screening and prevention strategies.

1302

FACTORS INFLUENCING HIGH RATES OF CATCH UP GROWTH AFTER EARLY CHILDHOOD STUNTING IN CHILDREN OF URBAN SLUMS OF SOUTHERN INDIA: A COHORT STUDY

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Malnutrition and stunting in early childhood is a major public health problem in less developed countries. A lack of long term cohorts leads to a paucity of data on factors that influence catch up growth in children not enrolled in supplementary feeding programs. Our study in an urban slum in southern India investigated catch up in growth in children after early stunting. The study group was a birth cohort of 452 children, www.astmh.org
followed intensively for three years, but at 7-8 years, 273 children were contacted in 2010. Data was collected using a structured questionnaire and anthropology. For analysis, the cohort was divided into categories of children who were ever stunted at 12, 24 and 36 months and those who were never stunted. Of available children, 189/273 (69.2%) were ever stunted, but more than 80% of the 189 showed catch up growth by 2010. The mothers of the ever stunted group were younger by 1.4 years (p = 0.009), shorter (p = 0.009) and weighed less (p = 0.02) than mothers of never stunted children. Another variable that predisposed to stunting was household debt (Crude OR 1.82, 95% CI 1.07-3.08). Ever stunted children were divided into 2 groups, persistently stunted (33, 17.5%) and children with catch up growth (156, 82.3%) at the current follow up. In univariate analyses, factors associated with catch up growth were having <3 children, use of sunflower oil, use of a ration card, schooling of child in an unaided private school and using liquefied petroleum gas as cooking fuel. After multivariate logistic regression analysis, the factor independently associated with catch up growth was use of a ration card (Adjusted OR 3.16, 95% CI 1.01-9.76). Our study shows remarkably high rate of catch up growth, which was associated with use of a ration card issued by the public distribution system, indicating that there is potential for governmental interventions to decrease malnutrition in poor urban communities.

1303

INFECTION ETIOLOGIES OF ACUTE MENINGITIS AND ENCEPHALITIS IN GEORGIA

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In Georgia, there are diverse etiologies of meningitis and encephalitis, including vaccine preventable agents such as mumps virus, varicella zoster virus (VZV), Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae type B (Hib), and other viral agents (e.g. Epstein-Barr virus (EBV), tick-borne encephalitis virus (TBEV) and West Nile virus (WNV)). Prevalence information regarding these infections in Georgia is limited. In October 2010, a hospital-based surveillance study was initiated to determine the incidence of infectious etiologies of acute meningitis and encephalitis; enhance laboratory capacity for the diagnosis of central nervous system (CNS) infections; determine antimicrobial susceptibility profiles; and describe the risk factors and clinical presentations associated with etiologic agents of CNS infections. Patients with suspected meningitis and encephalitis were enrolled from three hospitals in Tbilisi. Cerebral spinal fluid (CSF) and acute and convalescent sera were collected for bacterial culture and RT-PCR testing for HSV types 1 and 2, mumps virus, enteroviruses, VZV, S. pneumoniaea, Hib, and N. meningitidis. The diagnosis of WNV, TBEV, and EBV was conducted via commercial ELISA assays. As of 21 March 2011, 66 patients have been enrolled (23 adults and 43 children) and 61 CSF samples tested. Initial laboratory results indicate the frequency of HSV-1, enteroviruses and VZV to be 43%, 38% and 2%, respectively. For both TBEV and WNV, the frequency was determined to be 7%. Nine samples were positive for TBEV and seven samples were positive for EBV in 131 pairs of acute and convalescent sera. One S. pneumoniae case was cultured from CSF. These preliminary study results suggest the presence of a wide-spectrum of pathogens among patients with suspected meningitis and encephalitis. This surveillance study serves as a model for enhancing patient care through understanding disease prevalence, building laboratory diagnostic capacity and designing future syndromic surveillance projects in Georgia.

1304

CLINICAL MANAGEMENT OF DENGUE: A PHYSICIAN EDUCATION PROGRAM TO IMPROVE CLINICAL OUTCOMES, PUERTO RICO


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In 2007-08, the Centers for Disease Control and Prevention (CDC) Dengue Branch and the Puerto Rico Department of Health (PRDH) conducted a survey to assess physician’s knowledge of dengue and clinical management practices. The survey identified limited knowledge of warning signs for severe dengue and early signs of shock and non-standard treatment practices including use of corticosteroids and non-isotonic crystalloid solutions. A review of fatal dengue cases in 2007 corroborated these findings. In 2008-09, CDC Dengue Branch developed and pilot tested a physician training course to address the deficiencies identified by the survey and fatal case review. Focus groups and interviews were conducted with attendees of the pilot course to evaluate instructional process and content, and the course was revised accordingly. The course was approved by CDC and accredited by the Accreditation Council for Continuing Medical Education for 4 CME credits in February 2009. The Secretary of Health of Puerto Rico mandated that physicians take the training as a prerequisite for re-certification by 2013. To fully implement the case management course, 52 physicians were selected, trained and certified as Master Trainers. From February 21, 2009 to December 31, 2010, 55 courses attended by 8,301 of the 12,929 licensed physicians in Puerto Rico were conducted. Most physicians (6,294, 76%) were trained between September 1 and October 31, 2010 in response to another mandate from the Secretary of Health that all primary care physicians be trained immediately due to the increased number of dengue fatalities. An evaluation of the impact of training on clinical practices will be conducted in the Fall of 2011. Findings from this evaluation will be used to redirect continuing training efforts and to develop an online dengue clinical management course. Lessons learned from the implementation of this training initiative will be shared with dengue endemic countries planning to train physicians on the clinical management of dengue.

1305

PERFORMANCE OF STABILIZATION TUBES FOR EXTENDING TIME TO ANALYSIS OF COMPLETE BLOOD COUNTS FROM TRIAL PARTICIPANTS AT A RURAL FIELD SITE IN MALI

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Stabilization tubes (ST, Beckton Dickinson, Franklin Lakes, NJ) extend the pre-analytical stability of whole-blood (WB) specimens taken for CD4 measurements by utilizing a proprietary supplement targeted at

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cell surface membranes and clusters of differentiation (CD) on white blood cells (WBC). We hypothesized that this membrane-protective effect might confer specimen stability by acting on other membrane-bound cellular components. ST were assessed using twenty (n=20) WB specimens collected during a malaria vaccine clinical trial in Mali, West Africa. WB specimens were collected into ST and EDTA Vacutainer tubes for comparison, and complete blood counts (CBC) were conducted at day 0 and then every 24 hrs for 7 days thereafter. All measurements of WBC parameters deteriorated (> 10% erroneous or missing values) after 24 hours post-collection, while all red blood cell (RBC) parameters remained largely unchanged through 6 days post-collection. Data analysis revealed that ST do not provide stability of WB after collection in our setting. Further investigations validating and implementing novel technologies in the field are greatly needed to ensure quality specimens are analyzed in clinical research.

EVALUATING BLOOD CULTURES IN GUATEMALA AFTER IMPLEMENTATION OF A DEDICATED PHLEBOTOMY TEAM

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Blood cultures (BCs) are important diagnostic and surveillance tools to identify invasive bacterial diseases. In January 2008, we established automated BCs at a rural hospital in Guatemala and provided frequent trainings, job aids and all supplies and materials. After the first year of implementation, we found poor adherence to protocols and high contamination rates. In August 2009, we implemented a dedicated, round-the-clock phlebotomy team. To determine whether this intervention decreased contamination rates and improved pathogen isolation, we conducted segmented regression analysis of a monthly time series of BC outcomes from the laboratory database. A blood culture was defined as one or two blood culture bottles filled with blood taken from the same site. We collected data on 2,140 BC prior to intervention (January 2008-July 2009) and 1,525 blood cultures after the intervention was fully implemented (October 2009 to September 2010). There was an increase in the median number of BC per month among children <10 years old (41 per month pre-intervention vs. 58 per month post-intervention, p<0.05) but not among persons aged 10 years and older (63 per month pre-intervention vs. 68 post-intervention, p=0.69). Among 858 BC in children <10 years old during the pre-intervention period, 14% of cultures were contaminated and 7% produced a pathogen, compared to 10% contaminated and 4% with a pathogen among 695 cultures post-intervention. Among persons aged 10 years and older, 3% of 1282 cultures taken pre-intervention were contaminated and 8% yielded a pathogen, compared with 1% contaminated and 4% yielding a pathogen of 830 cultures taken post-intervention. Segmented regression analysis showed no impact of the intervention on the contamination rates among children <10 years old (β= -15.3, p=0.13) or persons aged 10 years and older (β= -2.5, p=0.45). Similarly, there was no effect of the intervention on pathogen isolation rates among children <10 years old (β= -2.2, p=0.62) or persons aged 10 years and older (β= -3.6, p=0.55). The results from this evaluation suggest that contamination rates among young children are unacceptably high and may be preventing isolation of pathogens. In addition to strengthening efforts to reduce contamination during venipuncture, particularly of young children, a review of laboratory protocols and procedures may identify further opportunities to reduce contamination and identify meaningful pathogens.

LOOKING FOR GOLD, FINDING MALARIA: THE 2010 MALARIA SURVEILLANCE OF THE SURINAME GOLD MINING MALARIA CONTROL PROGRAM

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Malaria is endemic in the forested interior of Suriname. Since 2006 malaria cases have declined tremendously with dispersed foci remaining in the gold mining areas. Currently the majority of malaria infections occur among persons (ca. 15,000) engaged in small-scale gold mining and related activities. Because there were no formal health services in these remote areas, a Global Fund supported malaria program was initiated in 2009 to fill the gap. This control program builds further on a surveillance system established in 2006 as a pilot under the Medical Mission Malaria Program. The surveillance system gathers weekly information from the Tourtonne diagnostic and treatment facility in the Capital city Paramaribo and from a network of 18 home-based diagnostic and treatment facilities (Malaria Service Deliverers) in the gold mining areas. Malaria cases are diagnosed by blood film or rapid diagnostic tests. A descriptive analysis of preliminary surveillance data of 2010 will be presented and compared with data from the previous year. The system recorded 1548 cases of malaria in 2010 among gold miners; 1388 (90%) confirmed by microscopy and 160 RDT cases. This number represents a decrease of 31.5% from the 2259 cases reported for 2009. Plasmodium falciparum 39%, P. vivax 52%, mixed P. falciparum plus Pvivax (7%) and P. malariae (2%) were the species identified. Among the 1548 cases 961 (62%) were classified as imported from neighboring countries and 26 (2%) were of unknown origin. 40 cases were reported in pregnant women of which 8 were P. vivax relapse. The Annual Blood Examination Rate (ABER) was 52.83, Slide Positivity Rate (SPR) was 17.51 and the Annual Parasitic Index (API) calculated from autochthonous cases was 30 in 2010 compared to ABER 48.34, SPR 22.45 and API 39 in 2009. In comparison to 2009 a notable decrease in the number of malaria cases from gold mining areas was reported in 2010. Conveying the importance of adhering to appropriate preventive measures for malaria to the population at risk is mandatory for the decrease in malaria cases to be sustainable.

ADULT REFERENCE VALUES FOR COMMONLY USED BIOCHEMICAL AND HEMATOLOGICAL TESTS IN CENTRAL GHANA

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Laboratory results and clinical examinations, provide useful information in screening, diagnosing and monitoring of diseases. Interpretation of laboratory results depend on reference values obtained from apparently healthy individuals from the population they are intended to serve. The reference values obtained from healthy residents of the communities used for clinical studies will help in determining eligibility and assessing the safety of those participating in these studies. This study was aimed at establishing gender-specific haematological and biochemical reference values for healthy adults in central Ghana. A total of 625 adults between the ages of 18 and 60 were enrolled within Kintampo and its environs. The medians, 2.5th and 97.5th percentiles were determined for five haematology and five biochemistry parameters commonly considered during screening/enrolment and follow up monitoring of individuals who usually participate in clinical trials and also for health management.
The Clinical Laboratory and Standards Institute (CLSI) guidelines for defining reference values were used. Values established in this study were compared with those derived in the developed countries. The percentage of our healthy population which had out of range values based on the data from the United States and the United Kingdom were determined. The red blood cell (RBC) parameters (haemoglobin, haematocrit and RBC count), total leucocyte and platelet counts and urea values were significantly lower compared to values derived in the developed countries. Higher values were, however, obtained in our study for parameters such as Alanine aminotransferase, aspartate aminotransferase and total bilirubin. Up to 53% and 75% of the haematology and biochemistry values, respectively from our healthy population would have been declared as abnormal results if data for the developed countries were to be used. The results from this study support the need to establish reference values using individuals from the population it intends to serve. This will help reduce the inappropriate exclusion of potential clinical trial participants based on reference values derived in the developed countries.

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CLINICAL OBSERVATIONS OF HUMAN MONKEYPOX INFECTIONS IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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We describe the results of an observational study of the clinical natural history of human monkeypox (MPX) infections at the remote L’Hôpital Général de Référence de Kole in the rainforest of the Congo River basin of the Democratic Republic of the Congo (DRC). The cardinal observations from 244 subjects enrolled in the study are summarized. All subjects who were positive by pan-orthopox MBG PCR -- utilizing an onsite quantitative real-time assay (LightCycler) -- were also positive by a MPX-specific MGB PCR assay, suggesting that MPX may be the only poxvirus circulating in the area. Sequencing of MPX DNA from one subject’s scab showed only 17 nucleoside changes from the MPX Zaire 79 strain (collected in 1979) which was circulating during the WHO clinical characterization studies of 25 years ago when the case-fatality rate (CFR) was about 10%. This is the same isolate USAMRIID has used to develop non-human primate MPX models for drug and vaccine evaluation. The spectrum of disease severity in our study was broad as evidenced by lesion counts ranging from 2 to 8,617, viremia (by PCR) ranging from undetectable to 6.3 x 10^7 genomes ml/blood, and clinical status ranging from very mild to critically ill. The CFR to date is only 0.9% in subjects aggressively treated with antibiotics, antimalarials, antiparasitics, anti-inflammatory drugs, and IV fluids. A strong correlation appears to exist between maximum lesion count and maximum viral load. Low albumin and total protein levels, as well as elevated liver enzyme and alkaline phosphatase levels, were seen in nearly all cases. In one case, in which viremia was detected before the onset of clinical illness, the maximum viral load occurred before the appearance of lesions and coincident with the onset of symptoms. Fetal demise due to maternal transmission of MPX infection occurred in three of four cases of pregnancy. A high percentage of cases involved transmission within households. The severity of disease within families varied widely without discernable pattern. This observational study is expected to lead to future hypothesis driven studies.

ETIOLOGY OF UNCOMPLICATED FEBRILE ILLNESS AMONG CHILDREN 2 - 59 MONTHS OLD ATTENDING A PRIMARY HEALTH CARE CENTRE IN ZANZIBAR

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A better knowledge of non-malarial fevers is a critical component for improved case management of childhood fevers in the new context of low malaria transmission in Zanzibar. We therefore conducted a health facility based prospective cohort study of the relative frequencies of infectious disease etiologies of uncomplicated febrile illness in North A District, Zanzibar. We enrolled 650 children aged 2 to 59 months old presenting to a primary health care center with confirmed fever (axillary temperature ≥37.5°C) or history of fever within the preceding 24 hours. Patients were clinically managed according to the newly adapted Zanzibar Integrated Management of childhood illness (IMCI) guidelines, which include a Rapid Diagnostic Test (RDT) for malaria. Naso-pharyngeal and rectal swabs were collected for PCR-detection of respiratory tract infection and diarrhoeal etiologies. Further, full blood count, malaria microscopy, C-reactive protein, chest X-rays, urine cultures with antibiotic susceptibility testing, rapid detection of pneumococcal antigen in urine and of Group A Beta-Hemolytic Streptococci from throat swabs were analyzed. Preliminary results from a sub-sample of 292 patients of whom 41% had measured fever show the following distribution of clinical diagnoses (a patient can have >1 diagnosis): 52% pneumonia, 50% upper respiratory tract infection, 22% diarrhea, 8% tonsillitis, 4% skin infection, 3% ear infection and 2% dysentery. After laboratory testing no RDT or microscopy confirmed malaria infection was found. Streptococcus A rapid test was positive in 8% of all patients. Chest x-ray was performed in patients classified as pneumonia according to IMCI (cough and/or difficult breathing and increased respiratory rate). Radiological pneumonia consolidation was verified in 5% of those with pneumonia as initial diagnosis. Urine culture from non-diarrheal patients showed significant growth in 8%. These preliminary results indicate that a majority of the fever episodes were related to respiratory tract infections and diarrhoea. However, most IMCI classified pneumonias could not be confirmed with chest x-ray. Further PCR analyses will provide insight into the viral and bacterial etiologies of these febrile episodes.
BRONCHOPLEURAL FISTULA

Researchers

Counseling for Medical Missionaries and Pre-travel Vaccinations, Prescriptions and

Overseas volunteers and researchers face unique risks related to their travel purpose and duration. We sought to characterize these travelers and identify whether they received appropriate vaccinations, prescriptions, and counseling on travel-related issues. Boston Area Travel Medicine Network is a research collaboration of five travel clinics in the greater Boston area that sees ~7,500 travelers per year. We evaluated characteristics of travelers who reported their reason for travel as “missionary/volunteer” or “researcher/student.” Between March 2008-July 2010, 15,440 travelers were seen in BATMN clinics. Of these, 1451 (9.4%) were missionaries/volunteers, 1216 (7.9%) researchers/students, and 65 (0.4%) reported both reasons. The median age of all 2,732 was 24 years (range 8-85), and 907 (33.2%) were male. The median travel duration was 21 days (range 1-1096). Among 4,035 destinations, the most common were Haiti (308; 7.6%), India (228; 5.7%), Kenya (195; 4.8%), China (190; 4.7%), and Tanzania (176; 4.4%). Documentation was available for up to date vaccination status, evidence of immunity, or vaccine receipt at the clinic visit for 1,466 (53.7%) for Td/Tdap, 1,835 (67.2%) for hepatitis B, 2,323 (85.0%) for hepatitis A, 1,088 (39.8%) for influenza, and 199/286 (69.6%) for meningococcus (among persons going to at-risk countries).

Commonly prescribed medications included ciprofloxacin (1599; 58.5%), azithromycin (590; 21.6%) and levofloxacin (50; 1.8%) for traveler's diarrhea and atovaquone/proguanil (850; 49.0%), mefloquine (174; 10.0%) and doxycycline (120; 6.9%) for those 1732 persons traveling to chloroquine-resistant malaria risk countries. HIV post-exposure prophylaxis was prescribed for 1,235/1,598 (77.3%) travelers, and 385/1,943 (19.8%) had documentation of tuberculin skin testing. Blood-borne pathogen counseling was documented for 1,235/1,599 (77.2%), evacuation insurance counseling for 1,243/1,878 (66.2%), and rabies or animal bite counseling for 215/1,940 (11.1%). Missionaries, volunteers, researchers, and students make up less than 20% of BATMN travelers. Although it is likely that not all of these travelers had direct patient contact overseas, there are still critical gaps in the vaccinations and counseling they receive.

The patient presented with left-sided empyema, which was drained with a chest tube under water seal. Sputum was positive for acid-fast bacilli, and anti-TB therapy and antibiotics were initiated. After two weeks, a persistent air leak remained and chest radiography showed failure of lung reexpansion. The chest tube was trimmed to 4 centimeters and the 5th finger of a plastic glove with both ends cut was attached to the end and lubricated with vaseline, which allowed for one-way exit of air and fluid. The tube was left in place for three months to create a chest window, after which the tube was removed, leaving an epithelialized passage between the pleural space and external environment. Scant fluid continued draining from the valve and the chest window during the treatment course but no other complications were noted. Bacteriological cure was confirmed by negative control sputum at two and five months. After 12 months the window had closed spontaneously, and the lung had completely reexpanded. Radiographs illustrate the entire clinical course. The method described here using a widely available resource—a non-sterile plastic glove—to make a modified one-way valve was successfully used for the treatment of tuberculous bronchopleural fistula and persistent pneumothorax. Ongoing drainage of chronic empyema and formation of a chest window is thus possible without advanced thoracic surgical intervention. The glove drain is worth considering in resource-limited settings for this challenging complication of pulmonary TB.

THE BURDEN OF CHRONIC HEPATITIS B IN IMMIGRANTS IN QUEBEC, CANADA: A POPULATION BASED STUDY

Immigrants have higher mortality from chronic hepatitis B (HBV) and hepatocellular carcinoma as compared to those born in Canada. Despite this disparity there are no screening programs to detect chronic HBV, and HBV vaccine is not routinely given to immigrants after arrival in Canada. This is because there is no population based data describing the burden of chronic hepatitis B in immigrants. To fill this gap we created a cohort of all cases of hepatitis B reported from 1991-2008 in Quebec through linking administrative databases. We linked the MADO (Quebec Reportable Disease database), the MICC (Quebec Landed Immigrant database) and the RAMQ (Quebec provincial health insurance and physician billing database). For incidence rate estimates, denominators for immigrants were obtained from the MICC database (N=757,650 newly arrived immigrants from 1991-2008); for non-immigrants, denominators used 1991, 1996, 2001, and 2006 Quebec census data (immigrants removed). Rates and rate ratios and 95% CI were calculated using the Poisson distribution. 13,889 cases of chronic hepatitis B were reported during the study period. Non-immigrant cases were older (mean age 43.4 vs 33.4 p <0.01) and were more likely male (69% vs 51%, p <0.01). The rate of chronic hepatitis B overall was 10 fold higher in immigrants as compared to non-immigrants [rate ratio; 95% CI = 10.0 (9.7-10.3)] and rates/100,000 person years (PY) were 73.9 vs 7.4. Rates were highest for immigrants from East Asia/Pacific [rate/100,000 PY 95% CI = 280 (268-293)], Sub-Saharan Africa [280 (262-298)], Eastern Europe [86 (77-94)]; they ranged from 38-42/100,000 PY for immigrants from South Asia, the Middle East/North Africa and Latin America. Immigrants are at increased risk for chronic hepatitis B and its associated sequelae, including potential transmission to close contacts. Immigrants would therefore likely benefit from screening for chronic hepatitis B and verification of hepatitis B immune status, so that appropriate treatment of chronic infection and vaccination of susceptible contacts can be provided.
CORRELATION OF MALARIA RAPID DIAGNOSTIC TESTING WITH CLINICAL-BASED ALGORITHM IN A RURAL VILLAGE IN UGANDA

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Uganda has the world's highest malaria incidence and mortality. The Engeye Clinic was created in 2006 as a U.S./Ugandan non-governmental organization based in the Ddegeya Village. In this resource poor setting lacking microscopes and trained technicians, rapid diagnostic testing (RDT) was initiated to confirm clinically suspected malaria. Issues facing this community include little to no use of mosquito nets, failure to complete treatment and/or use of paracetamol substituted for malarial treatment by village merchants. The purpose of this study was to evaluate the implementation of a clinical algorithm in a resource-limited setting for the diagnosis of malaria compared to RDT. Over a two week period in February 2010, 344 patients were assessed by the on-site clinician using a clinical algorithm for the diagnosis of malaria. This included fever, chills, sweats, headaches, muscle or abdominal pains, nausea and vomiting for greater than 48 hours with no other obvious cause. RDT was performed on patients meeting clinical criteria for malaria by obtaining whole blood samples using immunographic testing. Treatment for suspected malarial cases was initiated with artemether/lumefantrine based on weight and pregnancy/lactation status. 117 patients met clinical criteria for malaria diagnosis. All clinically diagnosed cases were positive when confirmed with RDT for the detection of parasite specific antigens for P. falciparum. The prevalence of malaria as a cause of presenting symptoms was 34% in this cohort. This clinical algorithm was found to be highly specific. The specificity and the positive predictive value of the clinical algorithm was 100% when compared to the RDT in this cohort. In patients without clinical criteria for a diagnosis of malaria there were no positive RDT results. It was determined that the clinical algorithm could be used by rural health care workers to accurately diagnose malaria as misdiagnosis leads to a delay in treatment causing an increased mortality and unnecessary prescription of malarial medications and increased drug resistance.

LYME DISEASE AND FILARIASIS • A WOLBACHIA CONNECTION: A CASE REPORT

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Health care providers must consider neglected tropical and regional endemic diseases. Lyme disease, commonly found in North America requires a diagnosis of erythema migrans with confirmatory serology. Lymphatic filariasis, endemic to Africa, is a neglected chronic disease that can be easily overlooked in immigrants coming to North America. We present a case of a 21 year old Liberian male complaining of one week of right knee pain and swelling during the summer. He had a history of malaria and filariasis. He immigrated to Albany, New York eighteen months ago as a refugee from civil war and was taking isoniazid and pyridoxine for a positive tuberculin test. He had no known tick exposure or trauma. He was afebrile and hemodynamically stable complaining of right knee discomfort, warmth, swelling and decreased range of motion. His white blood cell count was 4.7 with 52% neutrophils, 26% lymphocytes, 9% eosinophils and 12% monocytes. C-reactive protein (101 mg/L) erythrocyte sedimentation rate (73 mm/hr) and IgG level (4123 U/ml) were elevated. Knee radiograph showed joint effusion. Synovial fluid aspiration contained 116,500 cells/μL (95% neutrophils) but gram stain and culture were negative. Lyme PCR from synovial fluid was not submitted for analysis. Lyme C6 peptide was positive as was confirmatory Western blot which demonstrated IgG bands 18, 23, 30, 31, 39, 41, 58 and 93. Antifilarial IgG4 was positive indicative of past or chronic infection with filariasis. A course of Doxycycline was initiated for the management of both acute Lyme arthritis and chronic filariasis. Antifilarial therapy with Doxycycline was directed toward the symbiotic bacteria, Wolbachia associated with microfilaria. While it is possible our patient’s positive Lyme serology reflected cross reacting antigens to filaria; his clinical presentation was consistent with Lyme associated arthritis. This case is unique in that antimicrobial management of one endemic infection was useful in the management of a geographically separate pathogen.

STRATEGIES TO PREVENT MEASLES, MUMPS AND RUBELLA AMONG NEWLY ARRIVED ADULT IMMIGRANTS AND REFUGEES IN CANADA: A COST-EFFECTIVENESS ANALYSIS

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Adult immigrants are an unrecognized group at risk for measles, mumps and rubella (MMR). They have been over-represented in rubella outbreaks and most cases of congenital rubella (CRS) in Canada have occurred in infants born to foreign-born mothers. Despite this gap, MMR vaccine is not routinely given to adult immigrants after arrival in Canada. We performed a cost-effectiveness analysis to define the optimal strategy to prevent MMR in this population. We constructed a decision analysis (Markov modeling) to compare the cost-effectiveness of four preventive strategies using MMR vaccine, compared to no intervention (status quo). Interventions tested were: 1 and 2) Vaccinating all adult immigrants with either 1 or 2 doses of MMR vaccine without prior serotesting, or 3 and 4) serotesting for MMR then vaccinating those found to be susceptible to one or more of the diseases with either 1 or 2 doses of MMR vaccine. A hypothetical cohort of 250,000 newly arrived immigrants 18 years of age or older was modeled over a 20-year period. The expected number of cases of MMR, associated complications and costs (health care system perspective) for each of the strategies were calculated with 3% discounting for costs and clinical outcomes. Vaccinating all adult immigrants with 2 doses of MMR vaccine without prior serotesting saved 41 million Canadian dollars and avoided 6,362 measles cases, 9 measles deaths, 5,051 mumps cases, 8,181 rubella cases, 1 rubella death and 38 cases of CRS over a 20-year period, as compared to no intervention. Comparing both interventions involving vaccination without serotesting, the 2-dose strategy cost an additional $1.69 per person (total of $422,500) compared to the 1-dose strategy but prevented an additional 273 measles cases, 405 mumps cases, 273 rubella cases and 1 CRS. Both strategies that involved serotesting before vaccination were cost-saving compared to no intervention. However, both serotesting strategies were more costly and less effective than the vaccination-only strategies. All new adult immigrants should be given two doses of MMR vaccine without prior serotesting: this strategy is cost-saving, prevents individual morbidity and mortality, and decreases the potential for outbreaks.

DETERMINANTS OF ANEMIA AMONG YOUNG CHILDREN IN WESTERN KENYA

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Anemia among children in developing countries is often attributed to iron deficiency caused by low dietary iron intake. However, the causes...
infectious. Anemia prevalence (hemoglobin 8.3 mg/dL) 75%, vitamin A deficiency (retinol binding protein (RBP) 10 mg/dL) 30%, reported fever in the last 24 hours 27%, stunting (height-for-age z-score<-2) 30%, wasting (weight-for-height z-score<-2) 3%, sickle cell trait 17%, sickle cell disease (SCD) genotype 2%, heterozygous \( \alpha \)-thalassemia genotype 9%, in vivo association analysis, anemia was associated with iron deficiency, vitamin A deficiency, malaria, inflammation, fever, stunting, wasting, homozygous and heterozygous \( \alpha \)-thalassemia genotypes, age<30 months, male sex, and low socioeconomic status (SES) (p<0.05). In a linear regression, accounting for cluster design, the best fit model included TIR, RBP, malaria, CRP, SCD, homozygous \( \alpha \)-thalassemia genotype, male sex, age<30 months (R²= 0.59, p<0.0001). Age <30 months, homozygous \( \alpha \)-thalassemia genotype, and CRP modified the relationship between iron deficiency and hemoglobin. Fever, height-for-age z-score, height-for-weight z-score, and low SES were not significantly associated with homoglobin when included in the best fit model and did not confound the relationship between TIR and hemoglobin. Interventions designed to prevent anemia should utilize an integrated approach, ensuring optimal iron intake while also addressing malaria and other infections.

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COMPLICATIONS OF MONKEYPOX INFECTIONS IN HUMANS

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We report on the complications of monkeypox infections in humans as observed during a 4 year (2007-2011) study at the remote L’Hôpital Général de Référence de Kole in the rainforest of the Congo River basin of the Democratic Republic of the Congo (DRC). The study was conducted jointly by the Institute National de Recherche Biomedicale (INRB) and the US Army Medical Research Institute of Infectious Diseases (USAMRIID). Human monkeypox infections were first identified during the final stages of smallpox eradication when laboratory testing determined that some cases clinically presenting as smallpox were actually caused by monkeypox virus. The present study was conducted at one of the two previous WHO MPX study sites (1981-1986) staffed by the same Spanish medical order of Catholic sisters who have continued the treatment of MPX patients after others in the country stopped hospitalizing such cases. A total of 244 patients were consented and enrolled into our study. Generally, patients presented with fever, chills, sore throat, pox lesions, and general malaise, fatigue. The number of pox lesions ranged from two lesions to more than eight thousand lesions. Complications included death, coma and other neurologic manifestations, co-infections, infected wounds, secondary dermatitis, miscarriages, keratitis, staphyloma, and caseation of eye lesions. The case fatality rate was 0.9% in our study. Case histories with appropriate graphics, including photographs will be presented, when appropriate, to demonstrate findings.

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MID-UPPER-ARM CIRCUMFERENCE IS A USEFUL TOOL FOR ASSESSING NUTRITIONAL STATUS IN YOUNG CHILDREN WITH DIARRHEAL DEHYDRATION

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Undernutrition is implicated in over half of all child deaths related to diarrhea. Cohort studies which assess the relationship between diarrheal disease and nutritional status typically use weight-based anthropomorphic measures. However, such measures are not useful to study the acute nutritional changes induced by diarrhea because children with diarrhea are often dehydrated. Mid-upper-arm circumference (MUAC) is a cheap, simple tool often considered to be a better indicator of acute malnutrition than weight. MUAC measures musculature, a proxy for protein stores. If truly unaffected by intestinal water content, or dehydration, MUAC could be used to assess the acute nutritional changes induced by diarrhea. In February 2011, we initiated a case control study in Mirzapur (Bangladesh), Kolkata (India), and Basse (The Gambia) to determine whether diarrheal dehydration affects MUAC. A case, enrolled at a clinic, was defined as a child 0-59 months old with acute (<7 days) diarrhea (≥3 abnormally loose stools in the previous 24 hours) who had ≥1 of the following: sunken eyes, skin tenting, dryness, IV rehydration, or hospitalization, and underwent rehydration therapy. A healthy control matched for age and gender was enrolled at home within 3 days of case enrollment. Weight and MUAC were measured for each case and his/her matched control at enrolment and again 4 hours later. To date, 66 cases and 66 controls have been enrolled. Cases had substantial weight gain within 4 hours, which was significantly more than the controls within 4 hours [mean kg 0.29 (0.16, 0.42) vs. 0.10 (0.02, 0.19) p=0.02]. In contrast, the change in MUAC before and after rehydration in cases was comparable to that in controls measured at two time points 4 hours apart [mean cm 0.03 (-0.01, 0.07) vs 0.01 (-0.01, 0.04) p>0.09]. These preliminary results suggest that rehydration therapy results in weight gain but does not affect MUAC, thus providing a useful tool for epidemiologic studies.

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EMERGING BIOMARKERS FOR THE DIAGNOSIS OF SEVERE NEONATAL INFECTIONS APPLICABLE TO LOW RESOURCE SETTINGS

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Severe neonatal infections are one of the most significant causes of pediatric mortality, resulting in more than 500,000 deaths each year, of which 99% occur in low-resource settings. Compared to clinical algorithms, new point-of-care diagnostics that could distinguish neonates with or without severe infections may have potential to substantially improve the global management of severe neonatal infections. This review sought to characterize promising biomarkers for the diagnosis of severe neonatal infections. Biomarkers extensively reviewed elsewhere (procalcitonin, C-reactive protein, tumor necrosis factor-α, interferon-γ, and interleukin-6 and 8) were not re-reviewed. Hundreds of other biomarkers have been associated with “sepsis”; this review focused exclusively on biomarkers with published performance data for the diagnosis of severe neonatal infections. We identified infant diagnostic
performance data on 21 biomarkers: seven acute phase reactants (Serum Amyloid A(SAA), LPS Binding Protein(LBP), Inter-α Inhibitor Proteins(Iα-ip), Antithrombin, Soluble E-Selectin, Fibronectin); five pro-inflammatory cytokines (Interleukin-1α, Interleukin-1β, Interleukin-12p70, Interleukin-18, Granulocyte Colony Stimulating Factor(G-CSF)); two anti-inflammatory cytokines (Interleukin-10, Interleukin-1 Receptor Antagonist(IL-1RA)); five chemokines (Growth Related Oncogene α, Interferon-γ Inducible Protein 10(IP-10), Monokine Induced by Interferon-γ, Regulated upon Activation Normal T cells Expressed and Secreted, Monocyte Chemoattractant 1); one soluble cell surface marker (soluble intercellular adhesion molecule-1); and one molecule involved in triglyceride metabolism (apolipoprotein CII(apoC2)). Seven soluble biomarkers (G-CSF, IL-RA, IP-10, SAA, LBP, lαlpl, apoC2), compatible with point-of-care immunodiagnostics (defined as a concentration > 1ng/ml), emerged as promising candidates, with sensitivity and specificity generally > 90% (range 33 to 100%). These biomarkers seem particularly attractive for future prospective studies of diagnostics for severe neonatal infections.

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OUTCOME OF FOUR PREGNANCIES IN CONGOLESE WOMEN WITH MONKEYPOX INFECTION

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The outcomes of four pregnancies in women with clinically apparent, PCR-confirmed, community-acquired monkeypox (MPX) virus infections are described. During 2007 to 2011 we studied the clinical features of human MPX infections in Kole, the Democratic Republic of the Congo. 244 subjects were enrolled of which four were pregnant. The outcomes of these four pregnancies along with the maternal pex lesion counts and the PCR-confirmed viremia were carefully documented. This is the first report of intrauterine demise due to complications of human monkeypox. In Case 1, MPX viremia rose rapidly and abruptly upon cessation of fetal movement at the 16th week of gestation, some 24 days after onset of rash. Marked fetal hepatomegaly and peritoneal effusion (hydrops fetalis) were seen at necropsy. In Case 2 a spontaneous miscarriage occurred in a subject without significant viremia or remaining lesions at the 6th week of gestation, 22 days after rash onset. The third spontaneous miscarriage occurred at 7 weeks of gestation and the 10th study day in a mother with over 1,000 lesions and viremia exceeding 10⁶ genomes/mL of blood. (No pathology examination was performed and no determination of viral load was made on the aborted material for Cases 2 and 3.) The fourth pregnant subject was enrolled for observation as a healthy family member of an index MPX case. She was then noted to be about 14 weeks pregnant. On her second study day she was noted to have MPX lesions on her genitals. Her lesion count never exceeded 20 and she had a low level viremia by PCR. At her study day 75 follow-up visit (24 weeks gestation), the fetus was alive. Although the sample size is small, we observed a very high abortion rate in cases of maternal MPX infections, but death is not inevitable.

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QUASISPECIES VARIANT ANALYSIS OF A 2010 DENGUE 3 VIRUS FROM KAMPHAENG PHEt, THAILAND

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All four serotypes of dengue viruses exist as quasispecies. Quasispecies are described as a spectrum of variants (‘candidate genomes’), genetically linked through mutation, creating an interactive population where selection acts on the population rather than the individual variant. We explored an assertion of quasispecies theory that the fitness (ability to infect and cause disease) of a particular viral sequence is determined more by its freedom to mutate than by its ability to replicate. A quasispecies from dengue virus serotype 3 (DENV3) was cloned from a single mosquito collected within a cluster of human dengue infections (100 meter radius) in Kamphaeng Phet, Thailand, in 2010, to understand diversity and mutational effects apparent in the population. Sequences were compared with other published DENV3 sequences and maximum likelihood phylogenetic analysis revealed quasispecies populations removed from the baseline ‘consensus sequence’ diversity of human DENV3 circulating in Thailand. Mutational analysis showed a high proportion of nonsynonymous mutations and 2.8% of the population was evolving faster per site than average despite overall low diversity. Forty-four percent of the sequences were under positive selection while 19% were under purifying selection. Quasispecies analysis identified amino acid substitutions that have been reported to lead to phenotypic changes in viral like particle assembly, prM/E protein production, glycosylation and/or antibody binding ability. Other uncharacterized amino acid substitutions identified are predicted to be deleterious. The diversity of the quasispecies suggests there are variants with altered abilities to infect and disperse with overall diversity being constrained in the mosquito. An altered ability to infect or disperse will potentially affect how the population responds to selective pressures such as innate immunity and vaccine implementation.

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VALIDATION OF DENGUE SEVERITY PREDICTIVE ALGORITHMS DERIVED FROM PRIMARY CARE AND HOSPITALIZED CASES IN AN ADULT SECONDARY CARE COHORT

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Dengue is the most prevalent arthropod-borne infection worldwide. In well-resourced centers where diagnosis can be rapidly established, the next crucial step is to triage for appropriate care. Singapore has primarily adult dengue disease and recent epidemics have led to development of predictors to guide admission to secondary care. We validate three algorithms in a prospective cohort of 137 laboratory confirmed adult cases referred to a hospital-based dengue clinic. Cases that have already fulfilled severity criteria at presentation are excluded from analysis. First, the decision tree classifier developed from a febrile (<=72hrs) primary care cohort with laboratory-confirmed dengue fever, as reported previously; a cycle threshold of real time reverse-transcriptase polymerase chain reaction <=20.9 with positive dengue IgG at presentation, or plateau
count of <108 000/mm³. Reported sensitivity (Sn) was 78.2% and specificity (Sp) 80.2% in predicting a platelet nadir of 50 000/mm³. In our cohort, Sn/Sp=88.9%/66.7% in an identically defined subgroup (n=30), with no significant difference between previously published and our Sn/Sp. Second, comparison was made with a decision tree developed from a retrospective hospital-based cohort to predict dengue hemorrhagic fever (DHF) (Lee et al Trop Med Int Health. 2009 Sep;14(9):1154-9). Reported Sn/Sp=100%/46% using any of a history of bleeding, serum urea >4 mmol/L, or serum protein <=67g/L. In our cohort (n=115), Sn was significantly lower at 85.7% but difference in Sp at 49.4% was not significant. Last, the predictive equation for DHF using history of bleeding, serum urea, serum protein and lymphocyte proportion from the same cohort (Lee et al, J Clin Virol. 2008 May;42(1):34-9) had Sn/Sp=97.6%/60.3%. Our Sp was significantly lower at 32.2% but Sn was not significantly different at 100%. While our cohort was more severe than the hospitalized training cohort and the primary care cohort (24% vs 4% vs 2.6% DHF), it is reassuring that sensitivities remain high. Given the wide spectrum of dengue disease and varying presentations in different populations, a thorough exploration of the utility of prognostic algorithms taking into account population and clinical factors such as time to presentation will be required to safely triage dengue patients. We showed that the utility of predictors may vary even within the same country depending on source of patients.

RAPID DIAGNOSIS OF DENGUE IN A HOSPITAL-BASED COHORT

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Accurate and rapid dengue diagnosis is vital to triage and management. The World Health Organisation (WHO) proposed in 2009 an updated clinical definition of probable dengue replacing 1997 criteria for suspected dengue fever. Definitive laboratory diagnosis of dengue is not always possible, and newer methods such as testing NS1 antigen are undergoing evaluation. We prospectively enrolled 205 adult suspected dengue cases referred to the Communicable Disease Centre, Singapore to comprehensively evaluate methods for rapid diagnosis of dengue. Clinical and laboratory criteria were evaluated, including daily PCR, NS1 antigen, and IgM/IgG serology for those positive by PCR or NS1 on presentation. Confirmed dengue cases (n=142) were positive by PCR/NS1 or by IgM seroconversion by ELISA at 3-4 weeks. Non-dengue cases (n=20) were negative by PCR, NS1 and IgM ELISA in paired sera. Forty-three cases could not be assigned an acute dengue diagnosis because of a lack of paired sera or elevated IgM/IgG without seroconversion. The sensitivity (Sn) of PCR at presentation (median fever duration 5 days, range 2-9 days) was 70.4% and specificity (Sp) 100%. Median duration of viremia was 6 days (range 3-11 days). For NS1, Sn/Sp=89.4%/100%, with median duration of antigenemia of 7 days (range 2-10 days), significantly longer (p<0.001) than median viremic duration. Using <=5 days of fever as a cutoff for early illness, the Sn/Sp of PCR (n=84) was 88.9%/100% vs 51.4%/100% late in illness (n=78), compared to NS1 of 90.3%/100% early and 88.6%/100% late in illness. Only 2 cases (1.4%) were detected only by IgM seroconversion with negative results by PCR and NS1. WHO 1997 criteria for dengue fever had Sn/Sp=98.6%/20.0% while the recent 2009 criteria for probable dengue Sn/Sp=97.9%/20.0%. Laboratory diagnosis using NS1 antigen had consistently high Sn/Sp, with markedly improved Sn compared with PCR after day 5 of fever (p<0.001), and was positive for a mean of 1.1 days longer than PCR. Assessing seroconversion did not substantially increase the sensitivity of diagnosis in hyperendemic Singapore. Both clinical guidelines had similar test characteristics: very sensitive but with poor specificity in a cohort of referrals for suspected dengue.

GENETIC DIVERSITY OF DENGUE VIRUS IMPACTS TO THE DETECTION SENSITIVITY OF RT-PCR BASED METHOD, ONE CAUTION FOR METHOD DEVELOPMENT AND QUALITY CONTROL ASSESSMENT

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Dengue virus (DENV), transmitted by Aedes mosquitoes, causes the disease in 50-100 million people per year in tropical and subtropical regions worldwide. Four DENV serotypes (DENV-1 to -4) can cause infections ranging from asymptomatic or mild febrile illness to severe hemorrhagic disease. Various RT-PCR techniques have been developed for rapidly detecting and typing DENV. The rapid diagnosis allows early initiation of patient care and specific preventive health measures. RT-PCR is also a useful diagnosis tool for surveillance studies. Our laboratory has used the modified Lanciotti’s conventional RT-PCR method as one of the diagnostic tests for DENV since 1994. The method was evaluated and classified as an optimal method for DENV diagnosis and surveillance by an international External Quality Control Assessment (EQA). However, the analysis of PCR results of 13,532 dengue confirmed cases by ELISA tested over 11 years (2000-2010) showed 17-42% negative PCR results. Among these negative PCR results, 36% were from acute sera from patients within 0-4 days of illness onset, typically a viremic period with a high percentage of virus isolation. We tested 300 samples from patients with dengue confirmed by ELISA and with negative conventional PCR results with in-house TaqMan RT-PCR which was classified as a ‘need of improvement’ method by EQA as part of a quality improvement effort. One hundred and eighty samples (60%) showed TaqMan positive results including 129 DENV-1 (43%), 39 DENV-2 (13%), 4 DENV-3 (1.3%) and 8 dual infections of DENV-1 and -2 (2.7%). Sequences of conventional PCR primers binding sites of 9 TaqMan positive samples including 4 DENV-1, 3 DENV-2, and 2 DENV-3 revealed points of mismatch between primers and templates that likely affected the sensitivity of the detection. These data indicated that the genetic diversity of DENV impacts the sensitivity of RT-PCR based methods, a critical concern during method development and quality control assessment.

CONSIDERATIONS FOR CHANGING PRNT DENGUE 4 REFERENCE VIRUSES; SUB-OPTIMAL IMMUNITY TO DOCUMENTED INFECTIONS

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The plaque reduction neutralization test (PRNT) is considered to be the “gold standard” to characterize and quantify circulating levels of anti-dengue virus (DENV) neutralizing antibody. The PRNT is used to define the immunogenicity of dengue vaccine candidates, support dengue seroepidemiologic and pathogenesis studies. Despite numerous efforts to standardize the assay and normalize data to better compare data between studies and natural and vaccine infections, there are several sets of reference viruses around the world. The Armed Forces Institute of Medical Sciences in Bangkok utilizes DENV-1 (16007), DENV-2 (16681), DENV-3 (16562) and DENV-4 (1036) reference strains. In 2006 the dominate
serotype in circulation in Thailand was DENV-4. In cohort studies we observed poor or absent PRNT titers using the 1036 DENV 4, genotype 3 strain (originally isolated in 1976 in Indonesia) to documented DENV-4 infections. New candidate DENV-4 reference viruses were selected from isolates collected in the last 10 years. These viruses were tested using a bank of sera from documented DENV-4 infections including homologous sera from the individuals from which the strains were isolated. A candidate reference strain was selected based on PRNT titers achieved, low cross-reactivity, and the ability of the virus to produce large well-formed plaques. More than 300 samples were tested with the old and new reference virus. Geometric mean titers were increased 4.2 fold. Using the new reference virus enabled identification of additional inapparent infections in cohort studies and has enhanced our ability to characterize the DENV-4 immune response. This study illustrates the need to continuously monitor the performance of viral strains in reference assays. Furthermore, this data suggests that dengue viral evolution may have a profound effect on tests that utilize reference strains.

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SAFETY OF A RECOMBINANT LIVE ATTENUATED TETRAVALENT DENGUE VACCINE IN HEALTHY ADULT VOLUNTEERS

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Dengue (DEN) virus threatens over half the world's population, causing debilitating dengue fever, dengue hemorrhagic fever and dengue shock syndrome leading to over 20,000 deaths every year. DENVax is a tetravalent live attenuated dengue vaccine that is based on the DEN-2 PDK-53 genetic backbone. DEN-2 PDK-53 has been tested previously in humans and was found to be safe and immunogenic. Recombinant DENVax-1, DENVax-3 and DENVax-4 strains were generated in which the 
prM and E genes of PDK-53 were substituted with those of DEN-1, -3 or -4 viruses. These recombinant viruses retain the genetic attenuation markers present in PDK-53 and direct the immune response to the other three serotypes. A single center, placebo-controlled, randomized study assessing the safety, tolerability of tetravalent DENVax formulations was performed in Rionegro, Colombia, a high altitude area with no Aedes aegypti and no dengue exposure. One of two dose levels (low or high) of DENVax was administered subcutaneously or intradermally to healthy male and female subjects with no pre-immunity to flaviviruses. Two doses of DENVax or placebo were administered, separated by an interval of 90 days. Safety was assessed as the frequency and severity of adverse events through physical examination, injection site examination, lab examinations, and subject diary cards. Clinical laboratory assessments included serum chemistry, hematology and urinalysis. The safety data demonstrate that both tetravalent formulations were well-tolerated by either route of administration. To date, the most frequent adverse events were local reactogenicity at the injection site for both dose levels and both routes of administration. Systemic adverse events were mild to moderate headache, muscle pain, nausea and fatigue. There were no meaningful laboratory changes. This study highlights the safety of the tetravalent DENVax formulations in healthy adults. Further clinical trials to assess safety, tolerability, and immunogenicity in other age groups and in dengue exposed individuals are being planned.

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DEVELOPMENT OF A RECOMBINANT TETRAVALENT DENGUE VACCINES (TDV) THAT LINKS INNATE AND ADAPTIVE IMMUNITY

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We have previously demonstrated that the domain III of West Nile virus envelope antigen (EII) fused to flagellin of Salmonella typhimurium (STF2, a TLR5 ligand) is immunogenic and efficacious against lethal WNV infections in mice (McDonald et al., 2007, J. Infect Dis. 195, 1607-1617). To develop a tetravalent dengue vaccine, we have designed, purified, and evaluated similar and alternative flagellin-Ell fusion vaccine formats, which differ in the site of antigen attachment to the flagellin. These fusion proteins can be efficiently and economically manufactured in E. coli fermentation systems. Here we report immunogenicity results of recombinant dengue vaccine candidates in monovalent, bivalent, and tetravalent formulations. BALB/c mice were immunized s.c. three times at 2 or 3 week intervals, and bled at various times post boost. In an efficacy study, AG129 mice lacking receptors of types I and II interferons were immunized with two or three doses of a monovalent DENV-2 vaccine candidate, and challenged with 2,100 LD50 of DENV-2 (strain NGC). Serum neutralizing antibody titers were determined by 50% plaque reduction neutralization test (PRNT50). Survival rates, weight changes, and viremia, as measured by qRT-PCR, of infected mice were determined. Our results indicated that immunizations of BALB/c mice with these vaccine candidates at doses of 2-15 µg elicit robust homotypic neutralizing antibody responses. Furthermore, a monovalent DENV-2 candidate conferred partial protection against a lethal DENV-2 challenge and significantly reduced viremia and weight loss in infected AG129 mice. The DENV-2 candidate was also found to elicit high PRNT50 titers in rabbits. Finally, BALB/c mice immunized with tetravalent dengue flagellin-Ell formulations developed strong neutralizing antibodies to all 4 serotypes of DENV (GMTs of PRNT50 = 200 - 3000). In conclusion, VaxInnate flagellin-Ell vaccine candidates are highly immunogenic in mice and rabbits and are effective in protecting AG129 mice against a lethal DENV-2 challenge, thereby justifying further development of a TDV.

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IDENTIFICATION OF HOST FACTORS THAT INFLUENCE DENGUE VIRUS INFECTION IN HUMAN PRIMARY MONOCYTES AND MONOCYTE-DERIVED DENDRITIC CELLS

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Dengue virus (DENV) is a flavivirus in the family flaviviridae that infects up to 50-100 million people per year, with 2.5 billion people at risk. The burden of disease is significant, with a clinical primary infection manifesting as fever, rash, severe headaches, and intense myalgia and arthralgia that persist for approximately one week. Elucidating the interactions between host cell proteins and the dengue virus is critical to the development of targeted and effective antiviral drugs. Learning the details of these interactions will be essential to be able to rationally design drugs targeting viral proteins, or to identify compounds that will interfere with host processes critical to DENV infection. The first step towards this end is to identify which host proteins interact with the dengue virus in a clinically relevant system. Proteomic evaluations of host cells following
Genes related to antioxidant defense, such as glutathione S transferase, that the survival of mosquito cells is beneficial form up-regulation of mitochondria dysfunction occurs in both cell types. It was demonstrated usually end up with apoptosis. In spite, production of ROS resulted from cells are generally trivial compared to that occur in mammalian cells. However, the fate is distinct between the two types of cells in response to the infection. Cytopathic effects (CPE) in mosquito cells but not the described primary target of dengue virus infection, primary human monocytes and dendritic cells. We used a proteomics-based approach to identify host factors relevant to dengue virus infection in primary human monocytes and monocyte-derived dendritic cells. After infecting these cells with DENV serotype 2 strain 16681 we compared their proteome to that of uninfected cells using the Beckman Coulter PF2D system, a fluid-based system analogous to a 2D-gel that separates proteins by isoelectric point (pI) followed by hydrophobicity. After comparing infected cells to uninfected cells we found approximately 75 proteins that either increased or decreased in abundance by greater than 2.5 fold in the presence of DENV. These unidentified proteins were then subjected to Mass Spectrometry analysis. Proteins down-regulated in the presence of DENV in both monocytes and monocyte-derived dendritic cells were chosen for further analysis to elucidate their role in the pathogenesis of dengue virus.

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SEROTYPE-SPECIFIC DENGUE VIRUS CIRCULATION AND DENGUE DISEASE IN BANGKOK, THAILAND FROM 1973 TO 2010
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Since 1962, the Queen Sirikit National Institute of Child Health (QSNICH) and AFRIMS have cooperated in a mutual public health effort to accurately diagnose dengue infections including serologic determinations of antibody patterns and identification of dengue serotypes. The epidemiologic data included all patients admitted to the dengue ward of QSNICh with suspected dengue fever and dengue hemorrhagic fever who were subsequently proven to have dengue infection by serology or virus detection. Available data from 1973 to 1999 have been analyzed and published previously (Nisalak et al, 2003). We report on data for the expanded years from 1973 to 2010 including many more cases of DENV-4 infection than were observed previously. Findings that were reconfirmed from the previous report: 1) primary cases are increasing relative to secondary cases; 2) symptomatic primary cases were most likely due to DENV-1; 3) Primary non-infant hospitalized cases were less severe than secondary non-infant hospitalized cases. The mean age of DHF cases are noted to be increasing. In 1973-1982, the mean age of primary and secondary infection was 4.5 years and 8.0 years, respectively. In 2001-2010, it was 6.1 years and 8.0 years. These findings highlight the longitudinal epidemiology of dengue over a uniquely extended period of observation. Further spatial analysis is planned to elucidate transmission dynamics.

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THE ROLE OF ROS SIGNALING IN MOSQUITO CELLS THAT SURVIVE DENGUE 2 VIRUS INFECTION
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Dengue virus (DENV) is naturally transmitted by Aedes mosquitoes between humans and replicates efficiently in mosquito as well as in mammalian cells. However, the fate is distinct between the two types of cells in response to the infection. Cytopathic effects (CPE) in mosquito cells are generally trivial compared to that occur in mammalian cells that usually end up with apoptosis. In spite, production of ROS resulted from mitochondria dysfunction occurs in both cell types. It was demonstrated that the survival of mosquito cells is beneficial form up-regulation of genes related to antioxidant defense, such as glutathione S transferase (GST). The anti-apoptotic effect plays a role as the second defense system on protection of mosquito cells from DENV infection. It was eventually regulated by inhibitors of apoptosis (IAPs) that are the upstream regulators of caspase 9 and caspase 3. C6/36 cells with double knockdown of GST and IAP showed a synergistic effect on activation of caspasas, causing a higher rate of apoptosis (rate>20%) than those with knockdown of each single gene (~10%), after infection by DENV. Compared with mammalian cells, residual H2O2 after anti-oxidation in DENV-infected C6/36 cells may serve as the signal up-regulating the expression of IAP. Taken together, two defense systems including antioxidant defense and anti-apoptotic effects exist in mosquito cells; which were linked by ROS, i.e., H2O2 signaling.

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EPIDEMIOLOGY OF DENGUE IN MALAYSIA
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The first major epidemic of dengue fever in Malaysia occurred in 1973, and since that time dengue epidemics have become more frequent, and more virulent. We analyzed data collected by the Ministry of Health Malaysia between 2001-2010 and describe increasing incidence from 68.2/100,000 in 2001 to 159.7/100,000 in 2010, with a spike of 176.5/100,000 in 2008. Analysis of surveillance and notification data collected between 2005-2010 showed that the DHF/DSS:DF ratio was 1:19 in 2005 and 1:10 in 2010, with higher rates of severe disease in secondary versus primary infections. The mean age of DHF/DSS cases was 28 years. Age-specific incidence was highest in adults aged 20-29 years and incidence rates were higher in males, with a male to female rate ratio of 1.397 (95% CI: 1.390 - 1.404; p<0.005). Between 2005-2010 there was a shift to increased transmission in urban settings, with an increase in urban: rural rate ratio from 1.5 in 2005 to 2.0 in 2009, based on urban incidence rates of 170.4/100,000 compared to 98.7/100,000 in rural areas with an urban and rural rate ratio of 1.727 (1.719 - 1.735; p<0.005).

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AN ISLAND-WIDE DENGUE EPIDEMIC - PUERTO RICO, 2010
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Dengue, a potentially fatal febrile illness caused by four mosquito-transmitted dengue viruses (DENV-1-4), is endemic in Puerto Rico. In January, 2010, the number of suspected dengue cases reported to the Puerto Rico Department of Health/CDC passive dengue surveillance system exceeded the epidemic threshold. To characterize this epidemic, surveillance data were used to describe all reported cases. Suspected cases were patients with a serum specimen submitted for dengue testing. Laboratory-positive cases had (i) DENV identified via reverse transcriptase polymerase chain reaction (RT-PCR) in an acute specimen, and/or (ii) anti-DENV IgM detected in a convalescent specimen. Laboratory-negative cases had no anti-DENV IgM in a convalescent specimen and an acute specimen that was either RT-PCR-negative or not submitted. Indeterminate cases were RT-PCR-negative in an acute specimen and had