1

ANTIBODY RESPONSES IN RABBITS TO IMMUNIZATION BY THE SUBCUTANEOUS AND INTRADERMAL ROUTES WITH A METABOLICALLY ACTIVE, NON-REPLICATING (ATTENUATED) *PLASMODIUM FALCIPARUM* SPOROZOITE VACCINE

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The metabolically active, non-replicating (radiation attenuated) *Plasmodium falciparum* sporozoite (PfSPZ) vaccine was manufactured in compliance with current Good Manufacturing Practices (cGMPs). In the first Phase 1/2a clinical trial 4 doses of the PfSPZ vaccine will be administered subcutaneously (SC) or intradermally (ID) to volunteers. As part of the pre-clinical studies to support preparation of an Investigational New Drug (IND) application to the FDA, the PfSPZ vaccine was assessed in rabbits for toxicity and immunogenicity. Six groups of 24 rabbits (12 male and 12 females, total of 144 rabbits) were immunized SC or ID with 5 doses at 2 week intervals with the PfSPZ vaccine, phosphate buffered saline (PBS), or vaccine diluent. The results of toxicology studies will be reported elsewhere, but there was no apparent PfSPZ vaccine induced toxicity. There were three major immunological questions addressed, 1) was the PfSPZ vaccine immunogenic, 2) was there any evidence that the vaccine induced antibody responses against asexual erythrocytic stage antigens (PfMSP1 or PfEBA175) (a safety question), and 3) was there a difference in antibody responses between rabbits immunized by the SC and ID routes? The vaccine was highly immunogenic. In the intradermal group 2 weeks after the 4th dose of vaccine the OD 0.5 (serum dilution at which the OD was 0.5 by ELISA) was >160,000 against the Pf circumsporozoite protein (PfCSP); the end point titer by immunofluorescence against air-dried sporozoites (IFAT) was >100,000, and the inhibitory activity of the sera in the inhibition of liver stage development assay (ILSDA) at a dilution of 1:20 was 76%. There were no detectable antibodies against PfMSP1 or PfEBA175. The antibody levels by ELISA (anti-PfCSP) and IFAT (anti-PfSPZ) were higher in those rabbits immunized by the ID route as compared to the SC route. In the two control groups the background in the ELISA increased significantly after each dose of vaccine. Detailed assessment of the comparative antibody responses (SC vs ID) and the kinetics of the antibody responses will be presented. These data provide support for the clinical assessment of the PfSPZ vaccine by the intradermal route, and a foundation for the design of the antibody assays to be included in the first Phase 1/2a clinical evaluation of the PfSPZ vaccine, which will be discussed.

2

IMMUNITY INDUCED BY *PLASMODIUM BERGHEI* CSP EXPRESSION FROM VARIOUS CELLULAR LOCALIZATIONS AND DELIVERY BY INACTIVATED *ESCHERICHIA COLI*

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Inactivated bacteria (GeMI-VAX, Gene-mediated inactivation vaccine) are biological particles endowed with intrinsic adjuvant properties that can be used as expression and delivery vehicles for antigens, nucleic acids and drugs. The major advantage of this approach is that the inactivated bacteria have the ability to stimulate potent immune responses without the requirement of adjuvants. This approach is an attractive alternative to subunit vaccines as the inactivated bacteria serve not only as delivery vehicles for the antigen but also as the platform for expression of antigenic multimeric, multi-component (combination), and multi-valent vaccines. We intend to use the well-characterized rodent *Plasmodium berghei* malaria as a model system to study pre-erythrocytic-stage vaccine efficacy in mice using the circumsporozoite protein (PbCSP) as the target antigen delivered by GeMI-Vax bacteria. The PbCSP gene sequence was optimized by codon harmonization for optimal expression and folding in *E. coli*. Constructs were developed that target expression and localization of the PbCSP to the various compartments of the bacteria for delivery thus bypassing the requirement for antigen purification. The critical technical component of this approach is the efficient targeting of the PbCSP antigen to the appropriate bacterial compartments, here the outer membrane, periplasmic space, and lumen of the cytosolic space followed by their recognition, uptake, processing and presentation by antigen presenting cells (APCs) such as macrophage and dendritic cells (DC). The effect of the different antigen localizations within the bacteria on the resulting immune response is unknown and thus warrants detailed immunological analysis. We will report on the ability of PbCSP-containing inactivated bacteria to induce protective immunity against a live challenge and characterize humoral and cellular immune responses.

3

THE BIOCHEMICAL AND BIOPHYSICAL CHARACTERIZATION OF AN *ESCHERICHIA COLI* EXPRESSED *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE PROTEIN (CSP), A LEADING MALARIA VACCINE CANDIDATE

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The *Plasmodium falciparum* circumsporozoite protein (CSP) is probably a cornerstone of an effective malaria vaccine. RTS,S/AS02A (GSK, Inc.), incorporates the C-terminal half of CSP linked to the hepatitis B surface antigen formulated in the novel adjuvant AS02A. This malaria vaccine candidate has demonstrated a protective efficacy of 45% against clinical malaria in a phase IIb trial in 1-4 year old Mozambique children. Based on the demonstrated efficacy of this CSP vaccine, we desire to produce a recombinantly expressed CS protein for the development of vaccines with transmission blocking candidates, blood-stage vaccine candidates, or both. Currently, we have produced by a scalable process a full-length recombinant CS protein expressed in *Escherichia coli* using a synthetic codon optimized gene. rCSP is expressed in the cytosolic fraction of *E.coli* during high biomass fermentation in defined media (5L bioreactors). The *E. coli* expressed CS protein is captured by immobilized metal affinity chromatography prior to refold. The refolded CS protein is further purified by mixed mode hydrophobic interaction chromatography, anion exchange chromatography, and size exclusion chromatography. The purified refolded CS protein reacts with conformational dependent monoclonal antibodies with known *in vitro* inhibitory activity specific for the thrombospondin domain. Full biochemical characterization is currently in process, which includes reverse-phase HPLC analysis, atomic force microscopy, circular dichroism, sedimentation analysis, peptide and disulfide mapping, analytical size exclusion chromatography (SEC) with online multi-angle light scattering and quasi elastic light scattering, and electron-spray ionization mass spectroscopy. Our current results demonstrate that purified recombinant CSP has a mixed amino-terminus with over 96% of the protein accounted for by peptide mapping. Biophysical studies have shown that rCSP appears as a highly extended protein. Currently we are scaling-up the process for cGMP pilot production.

4

CHARACTERIZATION OF ANTI-AMA1 ANTIBODIES INDUCED BY AMA1-C2, A THREE-ALLELE COMBINATION VACCINE

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Plasmodium falciparum Apical Membrane Antigen 1 (AMA1) is a leading malaria vaccine candidate, but the highly polymorphic nature of AMA1 may lessen the efficacy of an AMA1-based vaccine. Previous phase 1 trials of AMA1-C1 (a mixture of FVO and 3D7 forms of AMA1) have demonstrated the vaccine induces growth-inhibitory IgG in humans, as judged by *in vitro* Growth Inhibition Assay (GIA). However, the IgG showed weak activity against heterologous parasite strains. To gain more extensive coverage of the antigenic polymorphisms, the AMA1-L32 form has been added (AMA1-C2). Although the AMA1-C2 vaccine may induce more cross-reactive IgGs, they may be less effective than strain-specific IgG induced by a single-allele AMA1 vaccine tested against a homologous strain. To address this question, rabbits were immunized with single alleles of AMA1 (FVO, 3D7, or L32) or AMA1-C2 and the avidity and biological activity of anti-AMA1 IgG were evaluated by surface plasmon resonance and GIA. Anti-AMA1 IgGs from rabbits immunized with single-allele AMA1 showed higher avidity to homologous AMA1 protein than to heterologous protein. Conversely, IgG from the AMA1-C2 group showed the same level of avidity to all three AMA1 proteins, which was equivalent to that of IgGs from single-allele groups tested against homologous AMA1. In agreement with the avidity data, both IgG from AMA1-FVO and AMA1-C2 immunized rabbits inhibited FVO parasites to the same extent and did so better than IgGs from either 3D7 or L32 immunized rabbits when antibody titers to AMA1-FVO were normalized. Similar results were obtained with 3D7 and L32 parasites in GIA. These results indicate that this three-allele combination vaccine, AMA1-C2, induces effective cross-reactive IgG against FVO, 3D7 and L32 strains and that these antibodies show similar avidity and growth inhibition activity to antibodies from single-allele groups tested against homologous strains. This study supports future AMA1-C2 vaccine development. The activity of anti-AMA1-C2 IgG against parasite strains not included in the vaccine will also be discussed.

5

THE FIRST GENERATION *PLASMODIUM FALCIPARUM* AMA-1 BASED MONOVALENT ADENOVECTOR VACCINE AND THE SECOND GENERATION BIVALENT ADENOVECTOR VACCINE EXPRESSING *PLASMODIUM FALCIPARUM* AMA-1 AND MSP1-42 ELICIT ROBUST FUNCTIONAL ANTIBODIES IN NZW RABBIT

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The US Military Malaria Vaccine Program and GenVec, Inc, in partnership with USAID and MVI, are developing adenovirus serotype 5-vectored vaccines designed to protect against *Plasmodium falciparum* (Pf) malaria, due to the demonstrated ability of this vaccine platform to induce strong cell-mediated immunity. Although adenovectors also induce antibody responses, it is not known whether these responses would be adequate to protect against blood-stage malaria. We have developed a two-component 1st generation vaccine NMRC-M3V-Ad-PfCA (AdCA), currently being evaluated in the clinic, where single constructs express either Pf (3D7) CSP (pre-erythrocytic) or AMA-1 (blood stage) antigens, and a two-component 2nd generation vaccine consisting of five antigens, where the bivalent blood stage component, M2V-Ad-PfMA (AdPf2), expresses both Pf (3D7) MSP1-42 and AMA1. NZW rabbits were adminstered two IM doses of $1x10^{10}$ pu AdCA or AdPf2 at 8 week intervals and growth inhibition assay (GIA) activity was evaluated with sera collected preimmunization and 4 weeks post boost. In separate studies, both AdCA and AdPf2 induced robust GIA activity predominantly associated with AMA1. The head-to-head comparison study reported here showed that the GIA activity induced by AdCA or AdPf2 vaccines were comparable, using either purified immunoglobulin (0.5-2.5mg/ml IgG) or polyclonal sera (20%). The AdCA-induced GIA was completely reversed by PfAMA1 protein. The AdPf2-induced GIA was largely reversed by PfAMA1 and completely reversed by PfAMA1+PfMSP1 proteins. The vaccine-induced GIA activity is effective against the homologous *Pf* 3D7 parasite but not the heterologous *Pf* FVO parasite. Significantly, the GIA activity induced by the adenovectors was >90% (2.5mg/ml IgG), a robust level of activity. Overall, data demonstrate that both monovalent and bivalent adenovectored malaria vaccine candidates can induce robust functional antibody responses, supporting clinical evaluation of these adenovectored malaria vaccines.

6

DEVELOPMENT OF A MULTI-ANTIGEN MULTI-STAGE ADENOVECTOR-BASED MALARIA VACCINE THAT INDUCES ROBUST T-CELL AND ANTIBODY RESPONSES

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More than 5,300 proteins are encoded by the *Plasmodium* parasite. Candidate subunit vaccines based on single antigens and traditional vaccine platforms have been poorly efficacious, indicating a need for new and effective vaccine technology platforms. Additionally, a major obstacle for the clinical development and fielding of a multi-valent vaccine is the cost associated with manufacturing the multiple antigen components. A practical solution is to construct and validate vaccine vectors capable of expressing multiple target antigens in the context of a single vaccine backbone. Adenovirus-based vaccines induce robust and protective immune responses in multiple disease systems and animal models, supporting their potential as a vaccine platform, but multi-valent advaccines have not yet been developed. We are developing a multi-antigen adenovector-based malaria vaccine that induces robust T-cell and antibody responses against both pre-erythrocytic and erythrocytic stages of the *P. falciparum* parasite life cycle. Our strategy is to incorporate three optimized pre-erythrocytic antigens (*Pf*CSP, *Pf*LSA1 and *Pf*Ag2) into a single E1/E3/E4-deleted adenovector vaccine to target the liver stage of the *P. falciparum* life cycle, and two optimized erythrocytic antigens (*Pf*AMA1 and *Pf*MSP1-42) into a single adenovector vaccine to target the blood stage. We conducted a systematic evaluation of promoters suitable for driving antigen expression in an adenovector for capacity to induce antigen-specific T cell and antibody responses and protective immunity in mice. We identified sites and configurations of expression cassettes that express all target antigens efficiently. In mice, trivalent pre-erythrocytic

and bivalent erythrocytic advaccines induced robust T cell and antibody responses against all antigens, with no apparent antigen interference. In rabbits, robust ELISA antibodies and GIA activity were induced by the bivalent advaccine. Our data indicate that multiple antigen expression adenovectors may be a cost effective way to induce and broaden protective immunity against malaria.

7

SAFETY AND TOLERABILITY OF A MULTI-STAGE, MULTI-ANTIGEN ADENOVIRUS-VECTORED *PLASMODIUM FALCIPARUM* MALARIA VACCINE, IN HEALTHY, MALARIA-NAÏVE ADULTS

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The global burden of malaria remains staggering, indicating the urgent need for a vaccine. The complex life cycle of the parasite, however, facilitates evasion of host immunity. To address this challenge, a multi-stage, multi-antigen prototype adenovirus (serotype 5)-vectored vaccine is under evaluation in a Phase1/2a trial, conducted by the US Military Malaria Vaccine Program in collaboration with GenVec, Inc, Gaithersburg, Maryland and the US Agency for International Development. Designated NMRC-M3V-Ad-PfCA, the vaccine comprises two adenovectors encoding the antigens PfCSP (expressed in sporozoite and liver stages) and PfAMA1 (expressed in sporozoite, liver and erythrocytic stages). The vaccine aims to induce strong cell-mediated immune responses as well as antibody responses targeting multiple stages of the parasite. In the first part of the study, two groups of volunteers (n=6/group) received either a single $2x10^{10}$ particle unit dose ($1x10^{10}$ per construct) or a five fold higher $1x10^{11}$ pu dose ($5x10^{10}$ per construct) of vaccine. The vaccine was generally well tolerated with most adverse events (AEs) mild to moderate in severity. One Group 2 volunteer experienced severe chills, myalgia and headache 8 hours post immunization that resolved rapidly. A greater number of volunteers in Group 2 compared with Group 1 reported vaccine related AEs and solicited systemic events in Group 2 tended to be of greater severity. There were no serious vaccine-related adverse events. The most common laboratory AE was a transient decrease in absolute neutrophil count noted on day 2 in most volunteers, associated with less marked decreases in absolute lymphocyte and platelet counts and a concomitant increase in monocytes. The most severe neutrophil decrease was 936/µl (grade 3) in the same volunteer experiencing multiple severe symptoms. Safety and immunogenicity data are being evaluated to down-select the optimal dose for the second part of the study where volunteers will receive two administrations of vaccine followed by sporozoite challenge to assess efficacy.

8

NEWLY ISOLATED MUTANTS OF DENGUE VIRUS TYPE 1 WITH DELETIONS IN THE 3′ NONCODING REGION SHOW HIGHER LEVELS OF REPLICATION *IN VIVO* IN MOSQUITOES

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The flavivirus 3′ noncoding region (NCR), which is approximately 400-750 nucleotides (nt) long, is divided into 2 regions on the basis of the difference in the conservation levels: (1) variable region (VR) and (2) middle and extreme 3′-terminal regions. The latter is highly conserved among strains and contains several sequence motifs crucial for viral RNA synthesis. In contrast, the function of VR in flavivirus remains to be determined. Flaviviruses, including dengue(DEN), Japanese encephalitis,yellow fever, and tick-borne encephalitis viruses, which possess nt deletions in the VR, have been reported since 1997. For example, we independently isolated 3 new DEN-1 strains, with 17-, 23-, and 29-nt deletions in the 3′ NCR VR, from dengue patients infected in Seychelles, Samoa Island, and Yap Island, respectively. Our previous analysis showed that DEN-1 VR is further divided into 2 subregions: a 45-nt-long 5′-terminal hypervariable region (HVR) and a 3′-terminal semivariable region (SVR). In the present study, we attempted to clarify the role of such a small natural deletion in the HVR as mentioned above. By using recombinant DENV-1 cDNA clones, we performed 3 new deletions/replacements as follows: deletion and replacement in the HVR, i.e., dHVR and rHVR, respectively, and deletion in the entire VR, i.e., dVR. dHVR and dVR exhibited reduced growth in mammalian cells as compared with the normal HVR and rHVR. In contrast, dHVR showed significantly enhanced replication in mosquito cells than other mutants.The amount of negative and positive viral RNA strands produced during synthesis increased in the dHVR-infected mosquito cells. The results of the reporter translation assay suggest that any VR mutations may not affect translation in DEN-1. These data indicate that dHVR mutants replicate to higher levels in mosquito cells. Further, to clarify the effect of deletion in HVR *in vivo*, we intrathoracically and orally inoculated the mutant viruses into *Aedes aegypti* and *A. albopictus*. The results indicate that dHVR mutants also replicate to higher levels in mosquito *in vivo*. RNA secondary structural analysis showed that HVR existed unbound to the loop structures and that dHVR mutants possessed a compact RNA structure. Thus, our data suggest that deletions in HVR attribute to high viral replication in mosquito and this might be the reason for the existence of such deletion mutants in nature.

9

MOSQUITOES PUT THE BRAKE ON EVOLUTION: EXPERIMENTAL EVOLUTION REVEALS SLOWER MUTATION ACCUMULATION IN MOSQUITO CELLS THAN VERTEBRATE CELLS

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Dengue viruses (DENV) are transmitted among vertebrates (humans) by mosquitoes, which may constrain their evolution by imposing constraints on optimal replication in either host if fitness tradeoffs occur. This hypothesis predicts that releasing DENV from alternating host replication will facilitate adaptation. To test this hypothesis, DENV was serially passaged either in one cell type to eliminate host alternation, or in an alternating passage series between vertebrate (Huh-7) and mosquito (C6/36) cells. After 10 passages, consensus mutations were identified and fitness was assayed by evaluating replication kinetics in both cells. Viruses allowed to specialize in single host cells exhibited fitness gains but fitness losses in the bypassed cells, and most viruses passaged in alternating cycles exhibited detectable fitness gains in both cells. Amino acid changes

common in both passage series suggested convergent evolution via positive selection. These results partially support the hypothesis that releasing DENV from alternating host replication facilitates adaptation.

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ANTIBODY DEPENDENT ENHANCEMENT OF DENGUE VIRUS INFECTION IN HUMAN DENDRITIC CELLS

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Dengue viruses (DV) are composed of 4 distinct serotypes (DV1-4) that cause 50-100 million infections annually. Durable homotypic immunity follows an infection but it may predispose a person to severe subsequent heterotypic infections, a risk that comes in part, from the immune response itself. Antibody dependent enhancement (ADE), a process best described *in vitro*, is epidemiologically linked to complicated DV infections, especially in Southeast Asia. This study reports for the first time the ADE phenomenon in primary human dendritic cells (DC), early targets of DV infection, and human cell lines bearing Fc receptors. Both immature and mature dendritic cells can support DV replication. We show that ADE is inversely correlated with surface expression of Dendritic Cell-Specific Intercellular adhesion molecule-3 Grabbing Nonintegrin (DC-SIGN) and requires FcRgIIa. Mature DC exhibited ADE whereas immature DC, expressing higher levels of DC-SIGN and similar FcRgIIa levels, did not undergo ADE. This is likely due to high expression levels of DC-SIGN on immature dendritic cells obscured ADE phenomenon. ADE results in increased intracellular de novo dengue protein synthesis, increased viral RNA production and release, and increased infectivity of the supernatants in mature DC. Interestingly, TNF-α and IL-6, but not IL-10 or IFN-γ, were released in the presence of dengue sera but generally only at enhancement titers, suggesting a signaling component of ADE. UV-irradiation of dengue virus abolished ADE and all cytokine secretions, suggesting that simple FcR cross-linking was insufficient for signalling. Similarly, FcRgIIa inhibition with monoclonal antibodies abrogated ADE and associated downstream consequences. DV versatility in entry routes (via FcRgIIa or DC-SIGN) in mature DC broadens target options and suggests additional ways for DC to contribute to the pathogenesis of severe dengue infection. Studying the cellular targets of DV infection and their susceptibility to ADE will aid our understanding of complex diseases and contribute to the field of vaccine development.

11

PRIMARY HUMAN ENDOTHELIAL CELLS SUPPORT DIRECT BUT NOT ANTIBODY-DEPENDENT ENHANCED DENGUE VIRUS INFECTION

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Microvascular plasma leakage is the hallmark of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). The precise molecular mechanisms leading to microvascular leakage are unknown, either dengue virus (DENV) or DENV-antibody immune complexes could affect the endothelial cell barrier through direct or indirect interactions that produce vasodilators and increase the vascular permeability. In this study, we analyzed the kinetics and consequences of DENV infection on human umbilical vein endothelial cells (HUVEC) using a molecularly cloned DENV2 in the presence or absence of human anti-DENV sera. Only 2% of HUVEC were infected by 96 hours post-infection as shown by FACS analysis. However, significant viral replication was detected as early as 24 hours post-infection using RT-PCR and plaque assays. Unlike monocytes/macrophages, HUVEC did not support antibody-dependent enhancement of infection due to a lack of FcγRI and FcγRII. Furthermore, direct DENV infection did not increase HUVEC apoptosis over basal levels. Since only a small percentage of endothelial cells were productively infected with DENV and this infection did not induce significant apoptosis, it is unlikely that direct viral infection is the major cause of the vascular leakage syndrome observed in DHF/DSS.

12

CANDIDATE GENE APPROACH TO IDENTIFY HOST GENETIC FACTORS FOR SEVERE FORMS OF DENGUE VIRUS INFECTION

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Dengue fever is acute febrile disease caused by infection of dengue virus. A recent surveillance revealed that up to 30% of the patients with dengue fever (DF) develop more severe form with hemorrhagic tendency, dengue hemorrhagic fever (DHF) or that with symptoms of profound plasma loss in addition, dengue shock syndrome (DSS), in the Southeast Asian countries. Multiple factors have been proposed for the development of these severe forms, and host genetic variations would contribute to pathogenesis to significant extent. To identify such variations associated to development of severe illness, we designed case-control study by collecting 743 patients with apparent dengue virus infection (114 patients with DF, 211 patients with DHF, and 418 patients with DSS, differentially diagnosed by WHO criteria) at two hospitals in southern part of Vietnam from year 2002 to 2005, and 193 healthy controls of the same Kinh ethnicity. At first, we employed pooled DNA genotyping which approximate allele frequency in the given population, for 85 short tandem repeat (STR) polymorphic markers physically linked to immune and inflammation related candidate genes. Comparison of one DHF pool composed of equal amount of DNA from 100 patients and two DSS pools (prepared similarly to DHF pool) with a control pool suggested the presence of alleles in different frequency at 22 out of these 85 loci. The differences in allele frequencies were then confirmed at 19 loci of them by genotype data of individuals who were included in the pools, indicating that the pooled DNA genotype was reliable in terms of the specificity of detection as a screening method. We extended the genotype analysis of these 19 loci for all available samples and found the association of alleles of 11 STR loci with either of DF, DHF or DSS vs. control. An allele of the STR locus located within *CD4* gene on chromosome 12 exhibited association with severe forms and significant genetic interaction with a disease-resistant factor linked to HLA class II genes, suggesting functional difference of helper T lymphocyte plays a role in the pathogenesis.

13

EVALUATION OF THE ROLES OF CD209 PROMOTER AND GENE POLYMORPHISMS IN PATHOGENESIS OF DENGUE DISEASE IN INDONESIA

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Dengue infection is a major cause of morbidity and mortality throughout the world, particularly in Indonesia. One of the human factors related to dengue virus (DENV) infection is the dendritic cell (DC), an antigen-presenting cell that functions to initiate the immune response by activating lymphocytes and stimulating the secretion of cytokines. DC-specific intercellular adhesion molecule (ICAM)-grabbing non-integrin (DC-SIGN;CD209), a C-type lectin specifically expressed by DCs, functions

as a pathogen pattern-recognition receptor and mediates dengue virus infection of human dendritic cells. In light of recent studies suggesting the association of polymorphisms in CD209 with individual susceptibility to several infectious diseases, we evaluated the association of DCSIGN1-336 (A/G) and -871 (A/G) polymorphisms with the development of dengue disease and investigated the degree of DC-SIGN neck region length polymorphism among a cohort of Indonesian adults with known dengue infection histories and clinical outcomes. Dengue cases in this study were confirmed by serology (positive IgM ELISA) and by serotype-specific RT-PCR. Individuals with undetectable anti-dengue IgG ELISA levels (<1.0) were included as controls. We found that the G variant and the wild type alleles of both DC-SIGN polymorphisms were equally distributed among individuals in the case and control groups, in contrast to a previous report from Thailand. However, the G allele of the variant DCSIGN-871 was associated with development of dengue hemorrhagic fever versus dengue fever (odds ratio:5.84, P=0.03) in the cohort we studied. A neck region of DC-SIGN plays a crucial role in tetramerization and support of carbohydrate recognition domain (CRD), thus influencing the pathogen-binding properties of this receptor. As reported in other Southeast Asia countries, most individuals had the wild type neck region repeated sequence variant (7/7), except in two individuals that harbored 7/8 and 7/6 variant (frequency :0.96%). These two polymorphisms did not appear to affect individual susceptibility to any particular dengue serotype.

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CHARACTERIZATION OF THE GENE EXPRESSION PROGRAMS ASSOCIATED WITH DISEASE SEVERITY IN ACUTE PEDIATRIC DENGUE INFECTION

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Dengue virus (DENV) infection in humans ranges in severity from asymptomatic infection to self-limiting febrile illness (DF) to severe and potentially fatal dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). The primary known risk factor for a more severe clinical course is secondary DENV infection, but much remains unknown about the pathophysiology of DENV infection. To better understand the molecular events associated with development of severe disease, we examined genome-wide transcript abundance patterns in peripheral blood mononuclear cells (PBMCs) of pediatric patients hospitalized with dengue in Managua, Nicaragua. Blood samples were obtained at presentation and for up to 6 successive days, and PBMCs were prepared within 2 hours and stored in Trizol at -80°C. We selected 29 patients sampled on fever day 4 (7 with primary DF, 7 with secondary DF, 9 who developed DHF, and 6 who developed DSS) and 8 healthy controls. RNA was purified and hybridized to exon-evidence based oligonucleotide (HEEBO) arrays. To characterize patterns of gene expression associated with different categories of DENV infection, we focused on the approximately 2000 genes with the greatest variance in transcript abundance and identified differentially expressed genes with a false discovery rate of 5%. There were many genes differentially expressed in healthy subjects versus those with DENV infection, and fewer specifically associated with the different disease classifications. An overlapping set of genes were more highly expressed in secondary DF, DHF, and DSS when each was compared to primary DF, and encoded proteins associated with the extracellular matrix and cell activation. These results suggest the presence of gene expression programs associated with secondary DF and DHF/DSS that will inform our understanding of the molecular events contributing to the development of severe disease.

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ANALYSIS OF THE EFFECTIVENESS AND SUSTAINABILITY OF METHODS FOR HOUSEHOLD WATER TREATMENT AND SAFE STORAGE

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Lack of sustained access to safe water creates tremendous burdens of diarrhea and other debilitating, life-threatening illnesses for people in the developing world. Point-of-use (POU) household water treatment technology (HWT) has emerged as an option that empowers people and communities without access to safe water to improve the quality of their water by treating it in the home. Several POU HWTs have been developed and evaluated to varying degrees, including boiling, POU filters, chlorine, combined chemical coagulant-disinfectant and solar (sunlight) disinfection in clear plastic bottles. With the possible exception of boiling, none have yet achieved sustainable, large-scale use, even though this is essential for effective HWT. The most effective, widely promoted and used POU technologies were examined and scored for laboratory and field performance over time based on the criteria of microbial reductions from water, daily water quantity produced, ease of use, cost, supply chain and sustained household use over time. By this analysis ceramic and biosand household water filters were identified most effective and having the potential to become widespread, sustainable POU HWTs for reducing waterborne disease and death.

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SUCCESSFUL PROMOTION OF WATER TREATMENT AND HAND HYGIENE THROUGH A PILOT CLINIC-BASED INTERVENTION FOR PREGNANT WOMEN SEEKING ANTENATAL CARE: MALAWI, MAY 2007-MARCH 2008

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Diarrhea is a leading cause of mortality among children in Malawi. Access to safe drinking water and improved hygiene are important for reducing morbidity and mortality from diarrhea. We evaluated a clinic-based intervention which promoted water treatment and hygiene to pregnant women through distribution of water storage containers, sodium hypochlorite water treatment solution, soap, and educational messages during antenatal visits. We surveyed 389 women receiving antenatal care at baseline before program implementation, and at follow-up 9 months later, to assess water treatment and hygiene practices, test stored drinking water for residual chlorine, and observe hand washing. We also surveyed non-pregnant female relatives and friends with children <5 years old to assess diffusion of the program into the community. We used McNemar's test to compare baseline and follow-up data. Program participants were more likely to know correct water treatment procedures (62% vs. 29%, p<0.0001), treat drinking water (61% vs. 2%, p<0.0001), and demonstrate correct hand washing practices (68% vs. 22%, p<0.0001) at follow-up than at baseline. They also were more likely to purchase and use the water treatment solution after free distribution (32% vs. 2%, p<0.0001). Relatives and friends who did not receive the program intervention were also more likely to know correct water treatment procedures (48% vs. 27%, p<0.0001), treat drinking water (25% vs. 2%, p<0.0001), demonstrate correct hand washing practices (60% vs. 18%, p<0.0001), and purchase and use water treatment solution (23% vs. 2%, p<0.0001) at follow-up than at baseline. This antenatal-clinic-based program is an effective new strategy for promoting water treatment and hygiene behaviors among pregnant women. Program participants purchased water treatment solution following free distribution, suggesting

that program benefits may be sustainable. Friends and relatives of program participants also exhibited improved water treatment and hygiene practices, which suggests that program impact extended beyond program participants.

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USE OF A NOVEL METHOD TO DETECT REACTIVITY TO STRUCTURED OBSERVATION FOR MEASUREMENT OF HANDWASHING BEHAVIOR

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Structured observation (SO), by having a trained observer in a subject's home to observe hand cleansing behaviors over several hours, is often used to evaluate handwashing behavior. We assessed reactivity to the presence of an observer, by measuring the increase in the number of times soap was used during a 5-hour SO, compared to the same time block on pre-observation days. In 6 Bangladeshi villages, we distributed bar soap containing motion sensors to participating households and returned 4 days later to conduct 5-hour SO. The motion sensors detected the number of times soap was used. We used repeated-measures analysis of variance (ANOVA) to assess for differences in soap use on pre-observation days. We compared the number of soap use events during the 5-hour SO to the mean number of soap use events during the same 5-hour time block on pre-observation days. Data from soap containing motion sensors was available from 45 households. The mean number of soap use events during the 5-hour time block on pre-observation days was 4.5 (range 0.3-10.6). ANOVA testing confirmed consistency in the number of soap use events detected during the 5-hour time block on each of the pre-observation days. During the SO, the mean number of soap use events was 6.3 (range 0-18.0) (p=0.012). Compared to the same 5-hour time block on pre-observation days, the number of soap use events increased during the SO by $\geq$ 20% in 28 (62%) households, including 10 (22%) households in which the number of soap use events increased during the SO by $\geq$ 100% (median increase 35%, range: 0-500%). We documented reactivity to the presence of an observer measuring handwashing behavior in 62% of study households. Pairing SO with soap containing motion sensors allows for the detection of reactivity. Among households shown not to be reactive, SO may provide useful information regarding soap use during critical times of pathogen transmission from hands. If reactivity cannot be ruled out, the validity of SO data for measuring handwashing behavior is questionable.

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ACCEPTABILITY AND USE OF ALCOHOL BASED WATERLESS HAND SANITIZER AMONG STREET FOOD VENDORS IN PILANI, INDIA

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Several studies have shown an increased risk of enteric infections among persons eating food from street food vendors. Street vendors often have limited access to water and soap for hand washing. Waterless hand sanitizer may be a useful tool to improve hand hygiene of street vendors, given its portability and proven impact on hand contamination. In a pilot study, we assessed acceptability and use of alcohol-based waterless hand sanitizer among Indian street food vendors in Pilani, Rajasthan, India. We completed a baseline survey of consenting street vendors working in Pilani. We taught street vendors to use the sanitizer at critical times, such as after using the toilet and before preparing food, and provided ample supply for a 2-week period. At follow-up, we queried self-reported sanitizer acceptability and use and measured the volume remaining in sanitizer dispensers. We conducted structured observations to assess sanitizer use among a subset of the street vendors. We enrolled 30 street vendors, and completed follow-up interviews with 28. Twenty-five (89%) street vendors reported the sanitizer to be an acceptable method for cleansing hands; of these 10 (36%) used $\geq$ 2 oz of sanitizer in the first week, suggesting ~ 9 uses of sanitizer per day. Those using <2 oz of sanitizer reported sanitizer use only at the beginning of the workday or after using the toilet. During observations of 10 street vendors, we recorded 81 critical times. Hand sanitizer was used in 27% of critical times. Also, hands were washed with soap (19%) or water without soap (38%), or they were not washed at all (16%). Those that did not find the sanitizer acceptable believed it contained acid that would harm the food, or found it difficult to use. Waterless hand sanitizer was acceptable to street food vendors in India, although the majority did not use it as much as prescribed. Further research is needed in larger study populations to confirm acceptability and to refine the intervention in order to maximize sanitizer use. Current cost of commercially available sanitizer is prohibitive for scaling up this potentially useful diarrhea prevention tool in a sustainable fashion in resource-poor settings.

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A GRAVITY-FEED HOUSEHOLD WATER PURIFIER DEVICE FOR USE IN THE INDIAN MARKETPLACE: LABORATORY AND FIELD EXPERIENCES

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A gravity-feed, household water purification device was developed for the Indian consumer marketplace, based on halogenated hydantoinylpolystyrene (HP) resin beads as the disinfecting medium. Laboratory studies using continuous challenge with suspensions of *E.coli* bacteria and MS-2 phage virus showed that mono-Br medium gave higher levels of effective disinfection for larger volumes of challenge water that did the di-Cl resin. A capacity of 3000L was established for an 18 gm HP Br resin bed cartridge, with a minimum 3 log reduction in *E.coli* throughout the useful lifespan. Prefilter characteristics were established in the laboratory to deal with the worst Indian source water (turbidity 40-50 NTU, hardness 1500 units, TOC 15 ppm). Functional prototype units (branded AquaSure) were placed in 80 households, and followed up at 15, 30, 45, 90 days, sampled for micro efficacy, and users were queried about sensory perceptions of the product water, mechanical operations of the device, use pattern adopted in the household (L/day, timing of water charging, source water). Results showed sustained efficacy (total coliform counts), a high level of acceptance of water quality (taste, odor, appearance) and satisfaction with manner and convenience of use. Two bacteriological failures were found to be due to mechanical issues (airlocks, faulty re-assembly). The study was expanded to 1000 homes for 6 month follow up of approximately 10%. There was sustained efficacy, and zero issues arising with sensory qualities of product water. Operational problems arose with airlocks, prefilter silt deposition, hardness precipitation, and penetration of the device by ants. Other prototype device configurations were tested around the same HP medium (a 2L pitcher, a 1L squeeze sports-bottle). Laboratory data indicated that HP provides unprecedented antimicrobial efficacy/life span combinations, when used in concert with prefiltration media (ceramics, carbon, non-woven polyester meshes).

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DIFFICULTIES IN SUSTAINING IMPROVED HANDWASHING BEHAVIOR, KARACHI, PAKISTAN

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Few data are available on the long-term effectiveness of handwashing promotion interventions. In a trial conducted in 2003 in low income squatter settlements in Karachi, Pakistan, households in 19 randomly selected neighborhoods that received free soap and weekly visits for 9 months encouraging handwashing with soap reported 53% less diarrhea than households in 9 control neighborhoods. We revisited trial participants 18 months after the intervention ended to evaluate soap purchase practices and diarrhea. In July 2005 field workers returned to households originally enrolled in the trial, collected information on recent soap purchases, inspected the households, and asked the mother of the youngest child to wash hands as she usually did. Study workers re-visited households weekly for the subsequent 14 months and collected information on household soap purchases and diarrhea. Neither health messages nor soap were provided. Among 710 households in the 2003 efficacy study, 560 (78%) were still present and agreed to participate in July 2005. Compared to controls, mothers in households originally assigned to the handwashing intervention were 2.4 times more likely to rub their hands together at least 3 times (55% vs. 23%, $p<0.001$), and 2.7 times more likely to lather their hands for at least 10 seconds (43% vs. 16%, $p< 0.001$) when asked to wash hands. In the ensuing 14 months, former intervention households purchased a similar amount of soap (2.3 vs. 2.2 g of soap per person per day $p=0.51$) and had a similar proportion of person-days with diarrhea (1.59% vs. 1.88%, $p = 0.66$) compared to controls. Although households that received free soap and 9 months of intensive encouragement to wash hands demonstrated better handwashing technique, their soap purchases and longitudinal prevalence of diarrheal was not significantly different from control households 2 years later. For handwashing to realize its disease prevention potential, development and evaluation of interventions to change long-term handwashing practices are essential.

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INTERNALISATION OF MICROBES IN VEGETABLES: MICROBIAL LOAD OF EXOTIC VEGETABLES AND THE RELATIONSHIP WITH DIFFERENT WATER SOURCES OF IRRIGATION

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Internalisation of microbes in vegetables is of great concern because internalised microbes are protected against removal by washing. The study was carried out to assess the rate of internalisation of microbes in exotic vegetables in Ghana and the relationship with pipe borne, stream and well water sources of irrigation. Standard microbiological methods were employed in microbial enumeration of 272 vegetables collected at the market level, and 60 vegetables (irrigated with pipe borne or stream/well water) collected at the farm level. Additionally, 30 samples each of pipe borne, stream, and well water used in irrigation, as well as 12 samples of soil on which the sampled vegetables were cultivated were analysed. The mean counts of vegetables were of the order $4.0x10^3$ cfu/g; $8.1 x10^2$ cfu/g; $2.0x10^2$ cfu/g; $3.5x10^2$ cfu/g for total bacteria counts(TPC), coliform counts (CPC), faecal coliform counts (FCPC) and yeast counts (YC) respectively. Some vegetables (cabbage) irrigated with pipe borne water had lower CPC and FCPC than their counterparts irrigated with streams/wells at $p<0.5$; generally, the rate of internalisation of coliform or faecal coliforms in vegetables using stream/well water occurred 2.7 times higher than using pipe borne water. Wide variations were observed in the microbial quality of the three types of irrigation water with stream water showing the highest contamination with a mean CPC and FCPC of $5.8x10^7$ cfu/ml and$1.6x10^7$ cfu/ml respectively. The mean CPC ($4.7x10^7$cfu/g) and FCPC ($1.8x10^6$ cfu/g) of soil samples were similar to those of stream water suggesting both sources exerted similar contamination rates of the vegetables. Generally, there were no significant variations between the rates of internalisation of microbes at the market and farm levels at $p<05$ indicating that internalisation of microbes in the vegetables mainly occurred at the farm level. The internalisation rate of microbes in vegetable in Ghana appears to be high. Safety practices associated with the commodity should therefore not be limited to external washing only. There is the additional need of heating the vegetables to eliminate microbes both externally and internally before consumption.

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A PHASE 1 TRIAL OF THE MALARIA TRANSMISSION BLOCKING VACCINE CANDIDATES PFS25 AND PVS25 FORMULATED WITH MONTANIDE ISA 51

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Pfs25 and Pvs25, surface proteins of the mosquito stage of the malaria parasites *P. falciparum* and *P. vivax*, respectively, are leading candidates for vaccines preventing malaria transmission by mosquitoes. This single blinded, dose escalating, controlled Phase 1 study assessed the safety and immunogenicity of recombinant Pfs25 and Pvs25 formulated with Montanide ISA 51, a water-in-oil emulsion. The trial was conducted at The Johns Hopkins Center for Immunization Research in Washington DC. The trial was designed to enroll 72 healthy male and non-pregnant female volunteers into 1 group to receive adjuvant control and 6 groups to receive escalating doses of the vaccines. Due to unexpected reactogenicity, vaccinations were halted and only 36 volunteers were enrolled into 4 groups: 3 groups of 10 volunteers each were immunized with 5 µg of Pfs25/ISA 51, 5 µg of Pvs25/ISA 51, or 20 µg of Pvs25/ISA 51, respectively. A fourth group of 6 volunteers received adjuvant control (PBS/ISA 51). Frequent local reactogenicity was observed. Systemic adverse events included two cases of erythema nodosum considered to be probably related to the combination of the antigen and the adjuvant. Significant antibody responses were detected in volunteers who completed the lowest scheduled doses of Pfs25/ISA 51. Serum anti-Pfs25 levels correlated with transmission blocking activity. This trial demonstrated that it is feasible to induce transmission blocking immunity in humans using the Pfs25/ISA 51 vaccine, but these vaccines were too reactogenic for further development. This is the first report of erythema nodosum associated with these antigens or adjuvant.

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A PHASE IB STUDY OF THE SAFETY OF MSP3-LSP CANDIDATE MALARIA VACCINE IN TANZANIAN CHILDREN AGED 12-24 MONTHS

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The merozoite surface protein-3 long synthetic peptide (MSP3-LSP), which comprises 3 major B-cell epitopes target of defence mechanisms and 4 T-helper cell epitopes from the *Plasmodium falciparum* protein MSP3, is

currently developed as a blood stage malaria vaccine candidate. Previous studies in European and African adults have shown that MSP3-LSP was safe and immunogenic, inducing antibodies able to kill *P. falciparum*. A double blind, randomized, controlled, dose escalation phase Ib field trial in 12 to 24 month old children has now been conducted in Korogwe, Tanzania to evaluate the safety and immunogenicity of MSP3-LSP adjuvanted by aluminium hydroxide versus hepatitis B Vaccine as a control (NCT00469651). Forty five children were enrolled and vaccinated according to 0, 1, 2 month dose escalation schedule. The results of safety, reactogenicity and immunogenicity profiles will be presented and discussed.

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RANDOMIZED, CONTROLLED, PHASE 1 STUDY OF THE SAFETY AND IMMUNOGENICITY OF THE AMA1-C1/ ALHYDROGEL® + CPG 7909 VACCINE FOR *PLASMODIUM FALCIPARUM* MALARIA, IN SEMI-IMMUNE MALIAN ADULTS

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AMA1-C1/ Alhydrogel® was safe and immunogenic in adults and children in malaria endemic areas, although the antibody response in children was lower than that seen in semi-immune adults. A Phase 1 study in malaria naïve adults showed that adding the novel adjuvant CPG 7909 to AMA1-C1/Alhydrogel increased antibody responses up to 14 fold. A randomized, controlled, double-blind Phase 1 clinical trial was conducted in semi-immune Malian adults to evaluate the safety and immunogenicity of AMA1-C1/ Alhydrogel + CPG 7909 in this population. A total of 24 healthy adults in Donéguébougou, Mali, aged 18-45 years were randomized 1:1 to receive either 80 µg of AMA1-C1/Alhydrogel or 80 µg of AMA1-C1/Alhydrogel + CPG 7909. A group of 6 participants was vaccinated first followed by a group of 18. Vaccines were administered IM on days 0 and 28, and participants were followed until 6 months after the final vaccination. AMA1-C1/Alhydrogel + CPG 7909 was well tolerated; all related adverse events were mild or moderate and no serious adverse events were observed. One volunteer in the CPG 7909 group was not revaccinated due to a mild hypersensitivity reaction occurring immediately after the first dose; this volunteer had a previously undisclosed history of allergies requiring corticosteroids. Preliminary immunogenicity data suggest significant enhancement of the antibody response in the group that received CPG 7909. Full safety and immunological data will be presented. This is the first use of the novel adjuvant CPG 7909 in an adult population in a malaria endemic area. A Phase 1 study in malaria exposed children is anticipated in Mali.

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RANDOMIZED, CONTROLLED, PHASE 2B CLINICAL TRIAL TO EVALUATE THE SAFETY, IMMUNOGENICITY AND EFFICACY OF WALTER REED ARMY INSTITUTE OF RESEARCH'S AMA-1 MALARIA VACCINE (FMP2.1) ADJUVANTED IN GSK BIOLOGICALS' AS02 VS. RABIES VACCINE IN 1-6 YEAR OLD CHILDREN IN BANDIAGARA, MALI

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The malaria vaccine candidate antigen FMP2.1 is a recombinant protein based on the 3D7 strain *Plasmodium falciparum* apical membrane antigen-1 (AMA-1). The purpose of this randomized, controlled Phase 2b clinical trial (NCT: 00460525) is to evaluate the safety, immunogenicity and efficacy of FMP2.1 formulated in GlaxoSmithKline's Adjuvant System AS02 in children in Bandiagara, Mali, West Africa. Four hundred healthy children aged 1-6 were randomized 1:1 to receive three doses of 50 µg of FMP2.1 in 0.5 mL of AS02 or rabies vaccine, 30 days apart. The primary efficacy endpoint is time to first or only clinical malaria episode (axillary temperature of ≥ 37.5 °C and parasitemia of $\geq 2500/mm^3$) occurring between randomization and six months after the third immunization. Secondary endpoints include incidence density of clinical malaria episodes during this same period, time to first or only clinical malaria episode caused by parasites with AMA-1 genotypes identical to the 3D7 vaccine strain with respect to designated polymorphic codons, and asexual parasite density. The primary safety endpoints are occurrence of solicited adverse events after each vaccination during a seven-day and 30-day surveillance periods and occurrence of serious adverse events throughout the two year study period. Titers of anti-FMP2.1 antibody will be measured by ELISA. Data from the time of enrollment through six months after the third immunization will be unblinded in July 2008 and analyzed while the study continues in a single-bind fashion. The study began in May 2007 and the primary efficacy surveillance period ended in February 2008. Safety, efficacy and immunogenicity results will be presented.

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PHASE IIB, RANDOMIZED, DOUBLE-BLIND TRIAL TO ASSESS THE EFFICACY, SAFETY AND IMMUNOGENICITY OF THE CANDIDATE MALARIA VACCINE RTS,S/AS01 IN KENYAN AND TANZANIAN CHILDREN

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The RTS,S/AS01 malaria vaccine candidate contains part of the *P. falciparum* circumsporozoite(CS) antigen with hepatitis B surface antigen (HBsAg) formulated in the GlaxoSmithKline proprietary AS01 Adjuvant System. This vaccine has been developed in parallel with the RTS,S/AS02 formulation, which has already demonstrated efficacy against clinical (32%) and severe malaria (49%) in Mozambican children aged 1 to 4 years. Ahead of the phase III programme, the vaccine will now be administered to larger numbers of infants and children in different countries. A phase IIb, randomized double-blind trial (NCT00380393), was undertaken in Korogwe, Tanzania and Kilifi, Kenya to assess the efficacy, safety and immunogenicity of RTS,S/AS01 when administered according to a 0, 1, 2 month schedule. 445 infants between 5 and 17 months of age were enrolled at each site. The results up to a cross-sectional survey occuring when children were between 5 months (latest vaccinees) and at most 10 months (earliest vaccinees) after the third vaccine dose will be presented.

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PHASE IIB, RANDOMIZED, DOUBLE-BLIND TRIAL TO ASSESS THE SAFETY, IMMUNOGENICITY AND EFFICACY OF THE CANDIDATE MALARIA VACCINE RTS,S/AS02 WHEN ADMINISTERED ACCORDING TO THE EXPANDED PROGRAM ON IMMUNIZATION SCHEDULE

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The RTS,S/AS02 candidate malaria vaccine is being developed for the routine immunization of infants and children living in malaria endemic areas. In order to facilitate administration, it is intended that RTS,S/AS02 will be integrated into the Expanded Program on Immunization (EPI) and will be delivered with routine childhood vaccinations against diphtheria-tetanus-whole cell pertussis (DTPw) and *Haemophilus influenzae* (Hib). In this phase IIb, double-blind trial, which was conducted at a single center in Tanzania (NCT00289185), randomized healthy infants received 3 doses of either RTS,S/AS02 or hepatitis B vaccines (*Engerix-B*™; GSK Biologicals) in co-administration with a multivalent DTPw/Hib vaccine (*TETRActHib*™; Aventis Pasteur) at approximately 8, 12 and 16 weeks of age. The safety and immunogenicity and efficacy results to 6 months after the last vaccine dose will be presented.

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PHASE II, RANDOMIZED TRIAL TO ASSESS THE SAFETY AND IMMUNOGENICITY OF THE CANDIDATE MALARIA VACCINES RTS,S/AS02 AND RTS,S/AS01 WHEN GIVEN ACCORDING TO DIFFERENT VACCINATION SCHEDULES IN CHILDREN IN GHANA

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The RTS,S malaria vaccine candidate contains part of the *Plasmodium falciparum* circumsporozoite(CS) antigen with hepatitis B surface antigen (HBsAg). When formulated in the GlaxoSmithKline proprietary Adjuvant System AS02, the RTS,S/AS02 candidate vaccine has demonstrated efficacy against clinical (32%) and severe malaria (49%), in Mozambican children aged 1 to 4 years. However, preclinical models and clinical data have demonstrated that the AS01 Adjuvant System is more immunogenic than AS02 and suggest that the AS01 formulation of RTS,S results in an improved efficacy profile in a laboratory challenge model. Studies are now underway to further evaluate these two vaccine formulations. This phase II, partially-blind trial, conducted at 2 centers in Ghana (NCT00360230), was undertaken to assess the safety and immunogenicity of RTS,S/AS02 and RTS,S/AS01 given either as three doses on a 0, 1, 2 months or 0, 1, 7 months schedule, or as two doses on a 0 and 1 month schedule, in children 5-17 months of age. The results, which will support the Adjuvant System and schedule selection for the planned phase III efficacy studies, will be presented.

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REGIME SHIFTS IN MALARIA INCIDENCE PATTERNS ARE RELATED TO CLIMATIC VARIABILITY, BUT MEDIATED BY INSECTICIDE TREATED NET USE

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Malaria is an important public-health problem in the archipelago of Vanuatu and climate has been hypothesized as important influence on transmission risk. Beginning in 1988, a major intervention using Insecticide Treated Nets (ITNs) was implemented in the country in an attempt to reduce *Plasmodium* transmission. It is unknown how this program may have modified the burden of disease, and whether there were any changes in malaria incidence that might be related to climatic drivers. Time series analyses of confirmed *P. falciparum* and *P. vivax* infections in the archipelago were analyzed during a 17 year period (January 1983 through December 1999). During this period, malaria dynamics underwent a major regime shift around May 1991, following the introduction of bednets as a control strategy in the country. By February of 1994 disease incidence from both parasites was reduced by at least 50%, when at most 20% of the population at risk was covered by ITNs. Seasonal cycles, as expected, were strongly correlated with temperature patterns, while inter-annual cycles were associated with changes in precipitation. Following the bednet intervention, the influence of environmental drivers of malaria dynamics was reduced by 30-80% for climatic forces, and 33-54% for other factors. The Vanuatu ITN program represents an excellent example of implementing an infectious disease control program. The distribution was

undertaken to cover a large, local proportion (~80%) of people in villages where malaria was present. The successful coverage was possible because of the strategy for distribution of ITNs by prioritizing the free distribution to groups with restricted means for their acquisition, making the access to this resource equitable across the population. These results emphasize the need to implement infectious disease control programs focusing on the most vulnerable populations.

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PROTEIN-GLYCAN INTERACTIONS MEDIATE MALARIA PARASITE TRANSMISSION

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Glycans are essential macromolecules for diverse cellular processes, such as signaling, structural-support, tissue development, trafficking, and cell-cell interaction. During the course of evolution, several pathogenic microorganisms have exploited the ubiquitous yet tissue- and class-specific distribution of cell surface glycoconjugates as receptors for attachment and invasion. However, only recently has evidence for a "protein-glycan recognition strategy" for vector host-pathogen interactions emerged. Malaria transmission entails development of the *Plasmodium* parasite in *Anopheles* mosquitoes. Circumstantial evidence suggests that midgut and salivary gland oligosaccharides are important ligands for parasite adhesion; however, the identity of these glycans remains unknown. We have identified distinct glycosaminoglycan populations along the apical midgut microvilli and basal lamina of salivary glands of *Anopheles gambiae* and provide evidence of their *in vivo* role in ookinete and sporozoite invasion via RNAi and small molecule inhibitors. These data offer insight into the molecular mechanisms mediating parasite-mosquito interactions and further suggests that protein-glycan recognition strategies by different *Plasmodium* life stages in both the vector and mammalian hosts are conserved. This conservation could lead to the development of novel multivalent approaches to disrupting malaria transmission and pathogenesis.

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MODELLING THE POTENTIAL IMPACT OF ARTEMISININ COMBINATION THERAPIES AND LONG-LASTING DRUG COMBINATIONS ON MALARIA TRANSMISSION INTENSITY: A CASE STUDY IN TANZANIA

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Artemisinin derivatives used in recently introduced combination therapies (ACT) for *Plasmodium falciparum* malaria have the potential to reduce population-level transmission of the parasite through their gametocytocidal action. Indirect evidence from areas implementing ACT supports this but it is not clear whether the impact is limited to low-transmission settings. With the increased interest in malaria elimination this becomes a key question. We developed a mathematical model to predict the potential impact of ACT on transmission outcomes in six areas of varying transmission intensity in Tanzania. We also compared ACT with a longer-acting antimalarial. Rates of treatment, asymptomatic and symptomatic infection in these areas were estimated from cross-sectional surveys prior to introduction of ACT. Estimates of ACT impact on infectiousness were taken from analysis of clinical trials. ACT were predicted to have a higher impact on infection prevalence and rates of clinical episodes in lower transmission settings because a higher proportion of infections were symptomatic and treated. In the area with lowest initial slide-prevalence (3.7%) we estimated a 53% decrease in incidence of clinical episodes if 100% of current treatment was switched to ACT, compared to only 21% in the area with highest initial slide-prevalence (57.1%). In absolute numbers, however, the public health impact was greater in the highest transmission area, with 54.1 clinical episodes/100/yr averted compared to 5.0/100/yr in the lowest transmission area. An efficacious antimalarial with no gametocytocidal properties but a long prophylactic time was estimated to be more effective at reducing transmission than a short-acting ACT in the highest transmission settings. These results show ACT have the potential for transmission reductions approaching those achieved by insecticide-treated nets in low transmission settings. In higher transmission settings antimalarials with longer prophylactic times used as partner drugs with artemisinins or in different antimalarial regimens may maximise transmission impact.

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CONTRIBUTION OF EXPOSURE-REDUCING INTERVENTIONS TO THE GOAL OF MALARIA ELIMINATION IN ENDEMIC AREAS

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The persistence of malaria as an endemic infection and one of the major causes of childhood death in most parts of Africa has lead to a radical new call for a global effort towards eradication. With the deployment of a highly effective vaccine still some years away, there has been an increased focus on interventions which reduce exposure to infection in the individual and - by reducing onward transmission - at the population level. Here we explore the impact of the trade-off between reduction in exposure and the development of immunity on the dynamics of disease. Our model predicts that the benefits of reduced morbidity in the first 5-10 years after the intervention when most of the population will remain highly immune may be offset by a greater burden of disease decades later. However, the negative impact of having fewer immune individuals in the population can be counterbalanced by the simultaneous implementation of multiple highly-effective interventions for an indefinite period or concurrent use of a pre-erythrocytic stage vaccine or prophylactic therapy in all children up to 9 years of age. Thus a dynamic, evolving intervention program may secure substantial, stable reductions in malaria transmission.

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NETWORK METAPOPULATION MODELING OF MALARIA VECTOR CONTROL

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There has been recent emphasis on the necessity of integrating multiple mosquito management tools in order to strategically optimize current malaria control programs. Given the extensive heterogeneity in vector populations known to occur between proximal habitats, it is important to develop understanding of mosquito ecology at an appropriately small scale. With the significant advances in computing power over the last decade or so, parameter-heavy simulation models of mosquito populations have become increasingly popular. However, in order to facilitate real-world application, general conclusions that are extrapolated from these 'small world' dynamics must be amenable to scale up to community-level control programs. To this end, a novel framework for simulating mosquito populations is proposed. This approach combines properties of two distinct theoretical tools: metapopulation models and stochastic network models. The effects of small-scale colonization and extinction events on the persistence of insect metapopulations have been analyzed extensively. Concordantly, network models have become a popular tool for simulating

the epidemiology of infectious disease. In this analysis, colonization will occur across a network of connected nodes (representing larval habitats), where the connections are stochastic dispersal paths of adult mosquitoes. Local extinction events are a result of breeding site elimination and occur naturally or through human intervention. The effects of adult mosquito dispersal behavior and between habitat variability on vector densities are analyzed. Simulations elucidate how knowledge of these factors can enhance the efficacy of breeding site destruction in reducing the overall adult metapopulation density. The importance of including spatial aspects in projections of mosquito models is emphasized. Specifically, it is shown how mean field approximations tend to under-estimate the resilience of mosquito metapopulations to control through resource management. Although metapopulation extinction using this strategy alone is likely to be extremely labor intensive, simulations of modest efforts in breeding site elimination show significant reductions in adult mosquito densities. Hence, evidence to justify the use of this control strategy as part of an integrated mosquito control initiative is provided.

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DIRECT AND INDIRECT EFFECTS OF HIGH COVERAGE VECTOR CONTROL ON PREVALENCE OF MALARIAL INFECTION

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The benefit of sleeping in a house that has been treated with indoor residual spraying (IRS) or of sleeping under insecticide treated nets (ITN) for protection against plasmodial infection is well documented. This study investigated the direct and indirect effects of IRS and ITN use both singly and in combination, on risk of infection. The Bioko Island Malaria Control Project, in collaboration with the government of Equatorial Guinea introduced an integrated malaria control programme consisting of IRS of all houses, case management consisting of ACTs and definitive diagnosis, IPT and information and education campaigns in 2004. In 2007 vector control was intensified through island wide ITN distribution, in addition to ongoing IRS. Prevalence of infection with malarial parasites in children was monitored through representative annual household surveys. Using multi-level modelling, the effects of IRS and ITN use separately and in combination, both direct and indirect, were investigated. 95% of children were covered by at least one of the two vector control interventions. Analysis showed that risk of infection for a child protected either by the use of an ITN or by living in an IRS sprayed house was reduced by similar amounts relative to those who were unprotected (OR=0.78 and 0.79 respectively, $p<0.01$). The combined protection of an ITN as well as living in a sprayed house further reduced the risk of infection compared to those receiving only one intervention. Living in a neighbourhood with high vector control coverage provided an additional protective effect, independent of whether the individual slept in a sprayed house, or under an ITN (OR=0.97 per 1% neighbourhood coverage, $p<0.01$). The effects of ITN and IRS vector control strategies are additive. In areas of high transmission the use of dual vector control should be considered. High vector control coverage provides protection even to those who do not receive the intervention individually.

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RAPID INCREASE IN COVERAGE WITH LONG-LASTING INSECTICIDAL NETS IN AMHARA, OROMIA AND SNNP REGIONS OF ETHIOPIA

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To contribute to the rapid scale-up of malaria control interventions in Ethiopia, The Carter Center assisted in procurement and distribution of 3 million long-lasting insecticidal nets (LLINs) in three regions of Ethiopia (representing 81% of the country's total population) where there are also ongoing onchocerciasis and trachoma control programs. Distribution was accomplished by ministry of health and local administrative staff, with the assistance of local trachoma and onchocerciasis workers. Baseline and follow up representative coverage surveys in malarious areas in the three regions of Amhara, Oromia and SNNPR were conducted in 5, 708 and 2, 550 households, respectively, of which the majority were located below 2000m. The proportion of households in these areas possessing at least one net increased from 37.0% (95% confidence interval (CI) 31.1 to 43.3%) to 72.8% (95% CI 64.8 to 80.7%) in less than one year, while the proportion possessing at least one LLIN increased from 19.6% (95% CI 15.5 to 24.5%) to 68.3% (95% CI 59.2% to 77.5%). The mean number of LLIN per house increased from 0.3 (95% CI 0.2 to 0.4) to 1.2 (95% CI 1.0 to 1.5). The proportion of persons sleeping under an LLIN the previous night went from 15.3% (95% CI 12.0 to 19.2%) to 34.5% (95% CI 27.3 to 41.6%) overall, from 17.4% (95% CI 13.6 to 22.0%) to 38.9% (95% CI 29.8 to 48.1%) for children under 5 years, and from 18.9% (95% CI 14.0 to 25.0%) to 37.4% (95% CI 25.5 to 49.3%) for pregnant women. These increases in net coverage and use were statistically significant. To build further on these encouraging gains and decrease the burden of malaria, health extension workers, community ivermectin distributors (for onchocerciasis) and trachoma community workers will be identifying remaining gaps in net coverage and the factors associated with use or non-use of mosquito nets. Appropriate educational materials are being developed to intensify promotion of proper use and care of LLIN.

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IN VITRO ASSESSMENT OF *TAENIA CRASSICEPS* MOTILITY AND ITS APPLICATION TO THE STUDY OF ANTHELMINTIC TREATMENT IN NEUROCYSTICERCOSIS

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Neurocysticercosis is a leading cause of adult-onset seizures in the endemic regions. Although albendazole or praziquantel are currently used for treatment, these are partially effective and incite significant neuro-inflammatory reactions. To identify safer and more effective therapeutic agents we developed a quantitative and sensitive *in vitro* drug screening assay using *Taenia crassiceps* (Tc) metacestodes as a model organism. In vitro motility is a recognized measure of viability in Tc and other cestodes. However these assays are qualitative in nature. A macroscopic imaging system composed of a dissecting microscope and a CCD camera was used to quantitate the motion of Tc. Three or four Tc metacestodes were placed in each well of an 8-chamber Lab-Tek II slide and 4 wells were

then recorded in a single video frame with a a burst of 30 images was collected every 2 hours. The motion detected between each recorded frame was quantified and averaged. The inherent variability over 6 hr in this novel system was determined to be within 35% of the initial level of movement. Praziquantel, known to cause a decrease in metacestode motility, exhibited a dose-dependent decrease in motility. Compared to DMSO controls that induced a 12% and 17% increase in motility at 4 and 8 hr, respectively, praziquantel at 0.1 µg/ml in 0.01% DMSO produced a 70% and 76% reduction in average motility at the same time periods. Cyclosporin A (0.1µg/ml), a useful anti-inflammatory agent with reported anthelmintic activity, caused an average of 75% reduction in motility at 24 hours post-treatment, compared to a 15% reduction in the control wells. Both methotrexate and albendazole sulphoxide failed to show a reduction in motility after 24 hours post-treatment compared to controls. Direct video-enhanced quantitation of motility can be used to identify potential useful compounds that have effects on metacestode motility and viability. In addition, the sensitivity of this method allows reliable detection of anthelmintic activity at lower concentrations than previously reported.

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TAENIA SOLIUM CYSTICERCOSIS IN NATURALLY INFECTED PIGS: VIABILITY OF CYSTICERCI AND PERSISTENCY OF SPECIFIC ISOTYPE ANTIBODIES AND CYSTICERCAL ANTIGENS AFTER TREATMENT WITH OXFENDAZOLE

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The aim of this study was to assess the effect of treating cysticercosis infected pigs with oxfendazole (OFZ) on viability and clearance of cysticerci and the corresponding persistence of specific isotype antibodies (IgG_{total}, IgG1, IgG2 and IgA) and circulating cysticercal antigen (CCA). Isotype antibodies and CCA responses were measured by antibody ELISA (Ab-ELISA) and antigen ELISA (Ag-ELISA), respectively. Correlations were made between antibodies, CCA and the total number of cysticerci enumerated at necropsy. Forty pigs naturally infected with cysticercosis were randomly allocated into 2 groups: Treatment group (n = 20) was treated with OFZ at 30mg/kg orally while the treatment control group (n = 20) was not treated. Five uninfected pigs served as negative control. Pigs were humanely killed at 1, 4, 8 and 26 weeks post treatment (wkpt). Overall, the mean total cyst count in treated pigs was 2,904 ±5,397 (mean ± SD) while in the controls it was 6,235 ±6,705. Mean cyst viability was 5 ±11% (mean ± SD) and 97 ±4% in treated and control pigs, respectively. Results showed that OFZ killed muscular cysticerci over a period of 4 weeks but failed to kill cerebral cysticerci. Antibodies, CCA responses and clearance of dead cysts from the meat, depended on the cyst intensity of individual pigs at time of treatment since both antibody and CCA correlated with intensity of cysticerci at necropsy ($r = 0.441$, $P = 0.005$; $r = 0.654$, $P < 0.001$), respectively. IgG1 responses were the best indicator of treatment efficacy because they were predominant in both infected treated and control pigs and disappeared early after treatment. Both Ab/Ag-ELISA failed to detect cysts in the brain. Though dead cysticerci took some time (26 wkpt) to clear from the meat, treatment of cysticercosis infected pigs with OFZ should, in combination with other intervention measures be considered as an important, cost-effective measure in the control of taeniosis/cysticercosis because it interrupts transmission by killing cysticerci which would transform into adult tapeworms.

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EFFECTIVENESS OF HEALTH EDUCATION INTERVENTION TRIAL TO REDUCE PORCINE CYSTICERCOSIS IN NORTHERN TANZANIA

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A randomised community controlled trial was conducted to estimate the effectiveness of health education intervention in reducing the incidence rate of porcine cysticercosis caused by *Taenia solium* in Mbulu District, northern Tanzania, between April 2002 and July 2004. A total of 827 pig-keeping households from 42 randomly selected villages were included in the study. Baseline data on the prevalence of porcine cysticercosis based on lingual examination of live pigs, and pig-management and sanitation knowledge and practices based on questionnaire interviews and observations were collected. Twenty-one of the villages were allocated to the health-education intervention, developed with community participation; stratified randomisation was used to balance the village-level baseline prevalence of porcine cysticercosis. From July 2003 to March 2004 following the intervention, each participant household was given a sentinel piglet to raise. Knowledge about the transmission and prevention of porcine cysticercosis was improved by 43% in both groups when measured 10-12 months post-intervention. There was no improvement in observed practices in either group throughout the study period. However, the intervention had a significant reduction in the reported cases of household consumption of infected pork (a reduction by 20%). The intervention was associated with a considerable decrease in the incidence rate of porcine cysticercosis (incidence rate ratio 0.57) as measured by antigen-ELISA in sentinel pigs. Public education could therefore lead to a reduction of the risk of cysticercosis in humans.

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A NATIONAL MODEL FOR THE CONTROL OF A PARASITIC DISEASE: CYSTICERCOSIS IN MEXICO

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Cysticercosis is acquired by humans and pigs after ingesting *Taenia solium* eggs released by human tapeworm carriers, who acquire the infection after ingesting insufficiently cooked pork infected with cysticerci. The Scientific and Technical Advisory Committee of TDR/WHO endorsed a Disease Entry/Exit Strategy in 2002. In Mexico, necropsies showed 2% of human neurocysticercosis (NCC) between 1943 and 1973; this burden of disease can justify an 'entry' strategy. Official notification of NCC and of taeniosis in the National Health System shows decreasing trends for both diseases since 1997 and thus can be viewed as the basis for an 'exit' strategy. Also, neurologists indicate that fewer NCC patients now request medical services and, although pigs are frequently found in backyards in small communities, where they have access to human feces, presently it is very difficult to find infected pigs in communities, markets and slaughterhouses. This suggests that *T. solium* may be under control in Mexico, reflecting the following: 1) National and international recognition of research on cysticercosis in Mexico, where scientific publications shifted from pathology to clinical aspects, diagnosis and chemotherapy, later on to epidemiology and control, and recently to basic biology and prevention measures; 2) Measures towards control of *T. solium* that include official guidelines for the control and prevention of taeniosis/cysticercosis published in 1994 and re-edited in 2004; 3) Availability of modern diagnostic techniques, cestocidal drugs, certified neurologists

and radiologists and, in general, expanded medical and veterinary human resources and 4) Improvement of economic, social and health conditions in the country.

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KNOWLEDGE AND BELIEFS ASSOCIATED WITH EPILEPSY AND CYSTICERCOSIS IN BURKINA FASO

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Epilepsy severely impacts the lives of people in Burkina Faso where the disease is believed to be highly stigmatized. The aim of this study was to describe the perceptions of disease in general and of epilepsy in particular, to explore the attitudes and behaviors towards persons with epilepsy and to evaluate the knowledge of the participants regarding the link between human epilepsy and pig cysticercosis. Three villages near Ouagadougou were selected to represent three types of pig farming: Batondo where pigs are roaming, Pabré where pigs are tethered and Nyonyogo with very few pigs. As our objective was to conduct a pilot study, we did not draw a representative sample of the general population in those villages. In each village, 3 young men (15-40 years old), 3 elderly people (40 years old and older), 1 traditional healer, 1 health care provider and 2 people with epilepsy were selected for a total of 30 participants. Three different semi-directive interview questionnaires depending on the type of participants were conducted and analysed qualitatively. In Batondo, people defined "disease" as something that is handicapping and prevents normal work. In Pabré and Nyonyogo, "disease" was associated with pain and dysfunction. In all villages, epilepsy is believed to have supernatural causes that can be treated with traditional medicine. Epilepsy is also believed to be transmissible. In Batondo, people with epilepsy are forbidden from gardening and from going to public places, and they are not allowed to become iron smiths. Epilepsy is associated with emotional and socio-economic burdens in caretakers as well, resulting in further marginalizion of people with epilepsy. Only four of the thirty people interviewed mentioned parasites as a possible cause of epilepsy (pig cysticercosis): two healthcare providers, one schoolboy and an elderly person with epilepsy who had neurocysticercosis. Epilepsy is considered as a supernatural disease in Burkina Faso. People still have many prejudices that negatively influence their behavior towards people with epilepsy. Those prejudices and negative stereotypes call for better education and information on epilepsy and its causes.

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PREVALENCE OF EPILEPSY, CYSTICERCOSIS AND NEUROCYSTICERCOSIS IN BURKINA FASO

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Epilepsy severely impacts the lives of people in Burkina Faso where the disease is highly stigmatized. Epilepsy is more frequent in sub-Saharan Africa than in the developed world, and one possible explanation is the high prevalence of neurocysticercosis (NCC) in pig raising countries. NCC is a brain infection with the zoonotic tapeworm *Taenia solium*. The aim of this pilot study is to estimate the prevalence of different types of epilepsy, cysticercosis and NCC in three villages of Burkina Faso. Three villages near Ouagadougou were selected to represent three types of pig farming: Batondo where pigs are roaming, Pabré where pigs are tethered and Nyonyogo with very few pigs. A census was conducted and all households in Batondo and Nyonyogo and one house out of two in Pabré were sampled. One individual was selected at random in each household to participate in the study by answering a screening questionnaire for epilepsy. Individuals screening positive and villagers declaring they had epilepsy were examined by a physician trained in neurology. Those with confirmed epilepsy were offered a CT-scan of the brain. All consenting participants provided a blood sample for the detection of antigens to the larval stage of *T. solium* using an ELISA test. A total of 890 people were screened for epilepsy and 62 were examined by a physician. Among those, the lifetime prevalence of epilepsy in Batondo, Pabré and Nyonyogo was 5.2% (95%CI: 3.1%-8.1%), 3.3% (1.7%-5.8%) and 6.4% (3.4%-10.9%), respectively. Among all villagers with confirmed epilepsy (80), 75 received a CT-scan of the brain. Lesions suggestive of NCC at the CT-scan were significantly more frequent among people living in Batondo (48.7% (32.4%-65.2%), n=39) and Pabré (63.6% (30.8%-89.1%), n=11) than in Nyonyogo (8.0% (1.0%-26.0%), n=25). Prevalence of cysticercosis was higher in Batondo (12.6% (8.8%-17.4%)) than in Pabré (1.8% (0.6%-4.2%)) and Nyonyogo (5.2% (2.3%-10.0%)). NCC is an important preventable cause of epilepsy in areas of Burkina Faso where pigs are raised.

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COMBINED GENOTYPAGE AND *IN SILICO* COMPARISON STUDIES OF PIG TAPEWORM *TAENIA SOLIUM* MATCH WITH UNIQUE ETHNOGEOGRAPHY OF MADAGASCAR

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Taenia solium is the causative agent of Taeniasis and Cysticercosis. This parasite is widely prevalent in Latin America, Asia and Africa. In endemic areas, *Taenia solium* is one of the major public health and pig husbandry economic problems. Two distinct genotypes of *T. solium* exist, i.e., Asian and American/African genotypes. Cysticercosis is a public health issue in Madagascar. The prevalence rate of the cysticercosis is less than 10% on the coast (Mahajanga and Toamasina Provinces) but more than 20% in the highland (Antananarivo and Fianarantsoa Provinces) where the livestock of pork is more important. We have collected 13 samples from 5 of the 6 provinces of Madagascar. Two mitochondrial genes have been studied in samples of *Taenia solium* isolated from infected pigs: *cytochrome b* and *subunit I cytochrome C oxydase*. We have compared the sequences from Madagascar with others sequences from different endemic countries: China, Thailand, Indonesia, India, Ecuador, Brazil, Mexico and Tanzania. A Bayesian analysis of data was further used to estimate the divergence time for major clades. Among 13 samples from Madagascar, 11 samples have the same signature than the samples from Asia (China, Thailand, Indonesia and India) while 2 samples have the same signature than the samples from America (Ecuador, Brazil and Mexico) and from Africa (Tanzania). So, both genotypes of *Taenia solium*, Asian and African/American are present in Madagascar.The study of the dates of divergence of the samples from different countries allows us to establish a hypothetical scenario. The presence of both genotypes is fully compatible with the human migration and species of pigs present in Madagascar.

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LACK OF TYPE I IFN IN DENGUE VIRUS (DENV) INFECTED HUMAN BLOOD CELLS MAY ACCOUNT FOR INEFFICIENT IMMUNE RESPONSES DURING DENV INFECTION

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Different cells in blood have been reported to be target cells for dengue virus infection (DenV). We are interested the interactions of the blood pathogen dengue virus (DenV) with different populations of cells from human blood in order to initiate immune responses in the host. We analyzed the ability of the DenV to replicate and produce infectious particles in those cells and the patterns of gene and cytokine expression in those infected cells. While all the cells tested were able to support DenV replication, monocytes and plasmacytoid dendritic cells (pDCs) released the most infectious particles. Suggesting that those cell types could be target cells for DenV amplification in blood. Also, most cells tested released high levels of chemokines and proinflammatory cytokines very early after infection, but did not detect any type I IFN production by these cells. Additionally, we analyzed the maturational profile of dendritic cells (DCs) after DenV infection using an extensive qRT-PCR and multiplex ELISA analysis of genes and proteins involved in DC activation as well as IFN related genes. Ultimately we tested the ability of DenV infected DCs to prime T cells to release IFNγ and we observed a lack of IFNγ released by those T cells after co-culturing with DCs. Our results suggest that DenV induces partial activation of DCs due to the lack of type I IFN release and that may result in poor T cell activation and lack of strong Th1 immune responses during DenV infection. All our data suggest that there are distinct and coordinated roles of different blood cells that contribute to the establishment of DenV infection as well as the initiation and/or evasion of immunity to dengue virus in humans.

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INTRINSIC ANTIBODY DEPENDENT ENHANCEMENT OF DENGUE INFECTION IN PRIMARY HUMAN MONOCYTIC PHAGOCYTES AND CELL LINES

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Antibody dependent enhancement (ADE) of dengue viral infection via Fc receptors leads to an increase in the number of infected monocytes, macrophages, and mature dendritic cells, as well as the amount of total infectious virions produced. It is unknown whether there is also an increase in the amount of virions produced by each infected cell. We hypothesize that aberrant cytokine production during ADE infection results in an increase in virus production per infected monocyte. We tested this by infecting human blood mononuclear cells with DENV2-16681 in the presence or absence of either polyvalent pooled dengue immune sera (PHS) or monoclonal human anti-dengue antibodies (HuMAb), and assessed infectivity using flow cytometry and plaque assays. We found that in comparison to direct viral infection, ADE infection increased both the proportion of infected cells [0.26% (0.08-0.6) to 0.98% (0.17-3.18); n=11; $p<0.01$ by one-way ANOVA test] and the number of infectious virions produced into the culture supernatant [1,450pfu (325-8,250) to 52,500pfu (5,500-300,000); n=11; $p<0.05$)]. However, the fold increase in viral output is much larger than the fold increase in the percentage of infected cells, suggesting that ADE infections might also have resulted in elevated virus production per infected cell. This also holds true in preliminary studies using two pairs of HuMAb against either DII or DIII of dengue envelop. To prepare for investigation into the potential mechanisms underlying intrinsic ADE, we observed that virus production was significantly elevated by low concentrations of PHS in K562 and U937 cell lines, which have often been used in dengue research. [K562: 440,000pfu (210,000-2,580,000) to 2,000,000pfu (1,700,000-18,900,000); n=3; $p<0.01$); U937: 88pfu (25-20,250) to 37,500pfu (35,000-3,000,000); n=3; $p<0.05$)]. These results highlight the possibility of intrinsic intracellular mechanisms that regulate dengue virus production during ADE of dengue infection *in vitro*.

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A MOUSE MODEL FOR ANTIBODY-ENHANCED DENGUE VIRUS INFECTION AND DISEASE

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Many attempts have been made to model dengue virus (DENV) infection in mice, but no DENV mouse model had been shown to exhibit antibody-dependent enhancement of viral burden and disease severity. We have previously shown that interferon-α/β and -γ receptor-deficient (AG129) mice are susceptible to DENV infection with all four serotypes, exhibit viral infection in relevant cell types including macrophages and dendritic cells, and develop a lethal vascular leak syndrome when infected with mouse-adapted DENV2 strain D2S10. Using this model, we have also identified conditions in which prior infection with one DENV serotype or passive transfer of anti-DENV antiserum protected mice from secondary DENV challenge. Here, we report conditions for antibody-dependent enhancement of DENV infection and disease in AG129 mice. Passive transfer of naïve mouse serum followed by infection with a non-lethal challenge dose of DENV2 did not result in mortality, whereas 90% of mice given anti-DENV1 serum one day prior to infection died of a vascular leak syndrome within 4 days. This increase in disease severity was associated with significantly increased viral burden in lymph nodes, bone marrow, liver, lung, and small intestine, with a particularly notable increase in peripheral blood. Furthermore, pre-treatment with anti-DENV1 antiserum reduced the inoculum of DENV2 required for lethality by 100-fold, from 10^7 to 10^5 plaque-forming units. We have furthermore demonstrated that DENV serotype cross-reactive monoclonal antibody 4G2 is also capable of mediating ADE in mice, with lethality consistently observed in mice administered 5-80 ug of antibody. The effects of enhancing antibody on cellular tropism, cytokine production, and other infection parameters is currently under investigation. Additionally, this model is being utilized to assess the effects of specific antibody parameters on antibody-mediated protection and enhancement *in vivo.*

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PRELIMINARY DATA ON A POTENTIAL RHESUS MACAQUE MODEL FOR DHF/DSS

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The precise chain of events and mechanisms involved in dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS) remain ill defined. The reasons to the failure are a) the logistic difficulty to study the early events in humans and b) there has been to date no reliable animal model that displays DHF/DSS following experimental infection. Although macaque monkeys, either subQ or I.M. administration of dengue virus, have been employed in the past to monitor experimental infection with either individual dengue virus types or following sequential dengue serotypes 1 to 4, they do not show any clinical signs of DHF/DSS and consequently the studies utilizing the rhesus macaque model have therefore been limited to study the nature of the replicating virus and the nature of the virus specific humoral and cellular response. Based on the 1939 report by Gordon and Lumsden that the proboscis of *aedes aegypti* following penetration of the skin "seek out" either the capillaries

or pooled blood leakage from capillaries for engorgement and are thus likely to deliver virus directly to the vasculature, we hypothesized that the IV route may be more representative of natural dengue virus infection. We thus experimentally infected each of 3 rhesus macaques (RM) IV with 10^7 PFU of dengue virus type 2 grown in Vero cells. Of interest was that 3/3 RM have readily shown readily detectable to extensive signs of hemorrhage on the chest, legs, point of infection, inguinal sites, etc, between days 3-4 p.i. Signs of petechiae were observed on each of the 3 RM by day 4 p.i. Detailed studies including blood chemistries, coagulation parameters, flow cytometric analysis of platelet adhesion to neutrophils and CD14+ monocytes, lymphoid cell subset analysis, CBC, and a variety of virological and immunological assays were performed on each of these 3 RM. Highlights of the changes noted were decreased in PTT concurrent with a marked increase in D-dimer and creatine kinase by day 7, which normalized by day 14, while the liver indicator enzymes SGPT and SGOT did nor fluctuate dramatically during the same time. An increased frequency of platelets bound to monocytes and neutrophils was observed during the acute phase. These findings represent the first report of a potential nonhuman primate model of dengue virus infection that develops DHF which may facilitate the identification of the potential receptor of the virus and the cellular, molecular and virological mechanisms that cause human DHF/DSS.

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INCREASED DENGUE DISEASE SEVERITY IN NICARAGUA IS ASSOCIATED WITH A CLADE REPLACEMENT IN DENGUE VIRUS 2

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Dengue virus (DENV) is a significant threat to public health worldwide, with tens of millions of cases of dengue fever (DF) and ~500,000 cases of the potentially fatal dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) occurring annually. In addition to host factors, viral determinants can contribute to disease severity. Certain epidemiological studies have suggested that particular DENV strains may be more likely to cause severe forms of the disease than others. In Managua, Nicaragua, we observed a dramatic increase in disease severity between the 2005 and 2006-7 dengue seasons in two different studies. In a hospital-based study of dengue, multivariate analysis of clinical and epidemiological variables indicated that immune status, year of study, age, and sex were significant factors contributing to disease severity. Interestingly, when adjusted for age, sex and immune status, the odds ratio for the 2006 dengue season was 6.58 (2.32-18.6). In parallel, in a community-based cohort study of pediatric dengue, incidence of DHF/DSS among dengue cases rose from 0% (0/78) in the 2004 and 2005 dengue seasons to 13% (8/64) in 2007. To determine if viral genetic determinants were associated with disease severity, we generated full-genome sequence for a large number of DENV genomes collected from both the hospital-based and community-based cohort studies in Managua. The DENV genomes sequenced formed two phylogenetically and temporally distinct groups (ML bootstrap=100) that were different from but related to the previously described Asian/American genotype. Contingency analysis revealed a temporal association (2005 vs 2006-7 $p<0.0001$, 1-tailed Fisher's exact test) as well as a significant association with disease severity ($p<0.02$, 1-tailed Fisher's exact test). Comparison of genome sequences from the 2005 to 2006-7 dengue seasons identified a set of eight specific amino acids that appear to correlate with the two clades. These residues spanned both structural and non-structural genes in the genome, including the envelope and NS5 genes. These results strongly suggest that changes in the virus have occurred that either led to an increase its virulence or rendered it more resistant to neutralization by antibodies from a previous DENV infection; functional analyses are underway to investigate these possibilities.

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SPATIAL HETEROGENEITY IN THE FORCE OF INFECTION OF DENGUE IN THAILAND AND THE SPATIAL STRUCTURE OF PHASE RELATIONSHIPS IN MULTIANNUAL OSCILLATIONS

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The incidence of dengue and dengue hemorrhagic fever oscillates with a multiannual periodicity of 2-4 years. This oscillation shows phase shifts in space with Bangkok and central parts of Thailand ahead in phase of other areas of the country. The reason for this phase structure has not been identified. We show here that the force of infection, the per capita rate of acquisition, of dengue varies spatially across Thailand. The central parts of Thailand have larger forces of infection showing dengue is more efficiently transmitted there than in the northern and southern parts of the country. We find that the amount by which each provinces multiannual oscillations are either ahead or behind in phase in relation to other provinces is strongly associated with the force of infection ($p<0.01$). Those provinces with high forces of infection are ahead in phase of those provinces with low forces of infection. We use a metapopulation model of dengue transmission to show that spatial coupling of communities with different intrinsic transmissibilities of dengue by migration of humans between patches can lead to phase shifts in incidence, with the same qualitative pattern shown in the empirical data. Finally, we estimate the critical vaccination fraction of dengue in each province, and show that the fraction that must be vaccinated to stop transmission varies substantially within Thailand with some provinces requiring 10-15% less of their population vaccinated in order to achieve herd immunity.

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A UNIFYING FRAMEWORK FOR THE COMPLEX REGIONAL DYNAMICS OF MULTI-SEROTYPE DENGUE VIRUS TRANSMISSION

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Explaining the regional spatial-temporal dynamics of dengue virus transmission is one of the most important unanswered questions in developing improved intervention strategies. Co-circulation of four antigenically related but distinct virus serotypes combined with scarce serotype-specific case data create uncertainty in defining networks of transmission. We developed an empirical spatial-temporal framework that identifies patterns in circulating infection (PCI) across 72 Thai provinces for 22 years by examining super-annual oscillations in incidence of hemorrhagic fever (DHF) and spatial-temporal indices of virus transmission. Our PCI framework combines four analytical layers: (1) a space-time spectral analysis of the period of DHF oscillations, an index depicting recurrence of epidemics, (2) spatial-temporal assessment of R_0, an index of transmission intensity based on age of infection and population age structure, (3) spatial-temporal changes in spatial synchrony of oscillations, an index of spatial dependence in epidemic recurrence across provinces using time series Empirical Mode Decomposition, and (4) an assessment of the phase relationships in serotype coexistence patterns in DHF incidence

across Thailand. The PCI framework reveals rapid, systematic evolution of mixing among virus serotypes, explains super-annual oscillations of severe disease, and brings focus to fundamental indices of transmission that will inform improved disease prevention planning and operation. The analytical layers of the framework illustrate an effective paradigm for understanding complex dynamics of highly networked spatial-temporal disease processes and provide a unifying foundation across competing perspectives on dengue transmission. Our results indicate that a universal, one-size-fits-all strategy will not effectively prevent dengue. Successful dengue prevention requires an awareness and understanding of spatial transmission structures and will depend heavily on whether intervention strategies are geographically adapted and carefully monitored.

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ECOLOGICAL AND GENETIC RELATIONSHIPS OF THE FOREST-M FORM AMONG CHROMOSOMAL AND MOLECULAR FORMS OF THE MALARIA VECTOR *ANOPHELES GAMBIAE* S. S

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Anopheles gambiae sensu stricto, one of the principal vectors of malaria in Africa, has been divided into two subspecific groups, known as the M and S molecular forms. Recent studies suggest that the M form found in Cameroon is genetically distinct from the M form found in Mali and elsewhere in West Africa, suggesting further subdivision within that form. Cytogenetically the Forest-M form is distinguishable from the Mopti-M form. The Forest-M form is characterized as carrying the standard chromosome arrangement for six major chromosomal inversions, namely 2La, 2Rj, 2Rb, 2Rc, 2Rd, and 2Ru. We found no chromosome inversion polymorphism within the Forest-M form. Bayesian clustering analysis based on molecular form and chromosome inversion polymorphism describes the Forest-M form as a distinct population relative to the West African M form (Mopti-M form) and the S form. The Forest-M form was the most highly diverged of the *An. gambiae s.s.* groups based on microsatellite markers. The prevalence of the Forest-M form was highly correlated with precipitation, suggesting that this form preferred much wetter environments than the Mopti-M form. Chromosome inversions, microsatellite allele frequencies and habitat preference all indicate that the Forest M form of *An. gambiae* is genetically distinct from the other recognized forms within the taxon *Anopheles gambiae sensu stricto.* Therefore, association studies of important phenotypes, such as insecticide resistance and refractoriness against malaria parasites, should take into consideration this complex population structure.

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CROSS-SCALE PATTERNS OF PALM TREE INFESTATION BY TRIATOMINE BUGS (HETEROPTERA: TRIATOMINAE) IN AMAZONIA

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Ascertaining whether a given pathogen, vector, or host occurs at a certain site is the cornerstone of infectious disease epidemiology. Failure to detect the organism of interest where it actually occurs constitutes a serious but frequently overlooked pitfall. The standard response is to invest on sampling techniques that increase detection probability (p) until $p \approx 1$. We present an alternative approach developed by wildlife biologists and especially suited to the pervasive situation where no sampling technique is perfect; instead of aiming for perfect detection, we invest on improved sampling design. We studied *Rhodnius* spp. (Triatominae) occurrence on 298 *Attalea* spp. palm trees across Amazonia; these bugs, primarily associated with palms, are major vectors of Chagas disease. Repeatedly sampling (live-bait traps and manual searches) palms provided information on the probability of detecting the bugs when they were actually present. This knowledge of the detection process allowed us to estimate the biologically relevant probability that any given palm was infested (ψ). We quantified ψ as a function of environmental variables measured over three spatial scales: four distinct eco-regions within Amazonia, three landscape classes (forest/rural/urban) within regions, and individual *Attalea* palm tree attributes. We conclude that: *Attalea* infestation is widespread and frequent (42-75%) throughout Amazonia; bug colonies tend to be denser in regions with higher net primary productivity; triatomine populations can recover after forest clearance; and micro-scale features (palm stem height and the amount of organic debris present on each palm crown) significantly increase palm infestation probabilities (ψ). Our results suggest that disease surveillance should emphasize capacity building among decentralized vector control teams with accurate knowledge of the local conditions, and that peridomestic palm tree management could help lower Chagas disease transmission risk in Amazonia. Our methodological proposal has wide applications in infectious disease research, and may open new perspectives for the rational design of vector surveillance-control strategies.

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SCABIES: EMERGING IVERMECTIN RESISTANCE IN A NEGLECTED ECTOPARASITIC DISEASE

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Scabies, an infectious skin disease caused by infestation of the mite *Sarcoptes scabiei,* is particularly problematic in socially disadvantaged communities and institutional settings. Of the few treatment options for scabies, ivermectin is the only available oral agent. It is the treatment of choice for hyper-infested (crusted) scabies, is used increasingly for ordinary scabies, and has been identified as a promising acaricide for mass treatment in scabies endemic communities. However, reports of ivermectin resistance in *S. scabiei* raise concerns regarding the sustainability of such programs. It is therefore critical to define the molecular mechanisms of emerging ivermectin resistance. Candidate mechanisms include 1) Drug efflux, mediated by ATP-binding-cassette (ABC) transporters such as P-glycoprotein; 2) Alteration to drug targets, such as ligand gated chloride channels and 3) Metabolic detoxification, mediated by enzymes such as glutathione *S*-transferases (GSTs). A survey of a *S. scabiei* expressed sequence tag dataset resulted in the identification of several ABC transporter and GST genes of interest. To evaluate transcription levels of these genes, mites were obtained from crusted scabies patients and separated according to life stage and ivermectin exposure. qRT-PCR was performed using SYBR green for seven target genes, amplified in parallel with β-actin, allowing for normalisation. GSTs were highly expressed at all life stages of *S. scabiei,* with expression of ABC transporter genes comparatively low. Of note, P-glycoprotein and GSTs were significantly up-regulated in ivermectin exposed adult female *S. scabiei,* suggesting these molecules may be associated with the development of ivermectin resistance in scabies mites. These advances should assist the development of molecular based diagnostics for the rapid detection of ivermectin resistance in *S. scabiei*. Such tools will become increasingly important

to avert the scenario of widespread emergence of ivermectin resistance, particularly in light of proposed mass-treatment programs.

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POPULATION GENETIC STRUCTURE OF *GLOSSINA FUSCIPES* IN UGANDA

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Human African trypanosomiasis presents a substantial health risk in southeastern and northwestern Uganda. Previous efforts to control the parasite have met with variable success and current approaches propose to control the tsetse vector via the release of sterile insects or to control the parasite via the release of tsetse engineered to produce an anti-trypanosomal compound. We have undertaken population genetic studies to resolve the spatial scale on which geographically disjunct populations of tsetse (*Glossina fuscipes*) interact, and thus to understand the scale on which vector control methods must be conducted. We sampled tsetse at 16 sites across Uganda, including foci for both *Trypanosoma b. rhodesiense* and *T.b. gambiense*. Using data from both mitochondrial and microsatellite loci, we address the extent to which tsetse from both foci have interacted historically. In addition, we provide more detailed analysis of unexpected population differentiation previously identified within the southeastern focus. Information presented here will comprise baseline data for a five-year project, which will examine spatio-temporal changes in population boundaries and the extent to which *Wolbachia* may play a role in structuring tsetse populations.

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THE PHLEBOTOMINE SAND FLY FAUNA (DIPTERA: SYCHODIDAE) OF SIX *LEISHMANIA*-ENDEMIC SITES IN KABUL CITY, AFGHANISTAN

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Since 1976, there has been essentially no new data compiled on the composition of sand fly vectors in Kabul, despite an estimated 60,000-200,000 annual cases of cutaneous leishmaniasis (CL). In order to begin compiling data on the present composition of potential *Leishmania* vectors in Kabul, we conducted entomological surveys for sand flies in Kabul city in 6 urban districts where numerous cases of CL have been reported. CDC light traps were used and placed in the districts from August 2007 to May 2008. Nineteen different species of sand flies were identified. Of these 19 species, 10 are *Phlebotomus* species, including 5 species (*P. papatasi, P. bergeroti, P. sergenti, P. alexandri*, *and P. major*) which are confirmed vectors of CL and/or VL. The remaining 9 species identified are *Sergentomyia* species. *Phlebotomus sergenti*, a confirmed vector of CL (*Leishmania tropica),* was collected in high numbers (68%) relative to the other *Phlebotomus* species collected. *Phlebotomus papatasi* and *P. bergeroti,* vectors of CL (*L. major),* comprised ~13% of the *Phlebotomus* species collected. Another *Phlebotomus* species, *P. keshishiani,* is considered to be a possible vector of VL. This species comprised 7% of the collection. Based on these findings, the Phlebotomine fauna of this region of Afghanistan appears similar to that found in CL and VL foci in Pakistan and Iran, despite ecological and climatic differences. Regular monthly trapping in Kabul city is intended to comprehensively identify the sand fly vectors of CL and VL, their seasonal population trends, and reservoir associations, in order to better understand the disease cycles in this region.

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IMMUNITY IN *LUTZOMYIA LONGIPALPIS*: PUTATIVE GENES AND IDENTIFICATION OF A NONSPECIFIC ANTIVIRAL RESPONSE

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Lutzomyia longipalpis is the major vector for visceral leishmaniasis in Brazil. In an effort to identify molecular events important in vector-pathogen interaction we have sequenced ESTs of cDNA libraries from *L. longipalpis* gut RNAs (6 and 72 hours after blood feeding, and 72 hours after artificial infection with *L. chagasi*). The expression of some of these sequences with a potential role in insect immunity was analyzed. A defensin gene is expressed in late larval stages, increases in adults and is modulated by blood ingestion. MAP-Kinase, involved in the initial steps of defense peptide synthesis, and VATPase, with a possible role in midgut acidification and indirectly in *Leishmania* metacyclogenesis, have low expression levels in final stages of development and high levels in adults. Cactus, responsible for the activation of several immune-related genes in adult insects, and TGF-β, both with dual roles in development and immunity, have constant expression, indicating a possible regulation at the protein level. RNAi has recently arisen as a convenient way of performing functional studies in insects. To establish RNAi assays in *L. longipalpis*, we have transfected cultured cells with double stranded RNAs (dsRNA), using West Nile virus-virus like particles (VLPs) expressing luciferase as model. Luciferase dsRNA caused a lowered production of VLPs as expected. Surprisingly, we found that various unrelated dsRNAs, that included the *E. coli* β-galactosidase sequence, diminished the production of VLPs. A similar response was seen in shrimp, but this is the first report on non-specific anti-viral response triggered by ds-RNA in an insect cell line. Preliminary experiments, submitting naïve cells to conditioned medium from cells treated with dsRNA followed by VLP infection, indicate the presence of soluble factors involved in the anti-viral response. Characterization of these putative factors is underway. It will be of great interest to identify the mechanisms by which LL5 cells recognize dsRNA, and the signaling pathway that produces the antiviral response.

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USAMRU-K ENTOMOLOGY: CAPACITY AND CURRENT FOCUS

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The U.S. Army Medical Research Unit-Kenya (USAMRU-K) Entomology Department conducts basic and applied research on a broad array of disease vectors. This includes basic surveillance efforts for leishmaniasis, arboviruses, rodent borne viruses, and malaria as well as testing of novel attractants/repellents and novel control techniques for vector species. USAMRU-K also maintains multiple field research sites, molecular laboratories, and an entomology laboratory capable of conducting arthropod vector identification and diagnostic assays for disease detection. Based in an area of Western Kenya with high endemicity for numerous vector-borne diseases, USAMRU-K Entomology is uniquely positioned to perform cutting edge vector-borne disease studies and to attract collaborative efforts in vector biology research.

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WASTE TIRES AND RISK FOR DENGUE FEVER INFECTION IN BROWNSVILLE, TEXAS AND MATAMOROS, TAMAULIPAS

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Discarded tires provide an ideal aquatic habitat for *Aedes aegypti* and *Ae. albopictus* mosquitoes, both vectors of dengue virus, because of water holding capacity, dark color, and thermal insulation which protects against weather extremes. Waste tires in the U.S - Mexico border region are thought to be one factor contributing to sufficient populations of *Ae. aegypti* and *Ae. albopictus* mosquitoes to pose a risk of dengue transmission to nearby humans, yet tires are difficult to control. This pilot project measured the effectiveness of two interventions in Brownsville, Texas and Matamoros, Tamaulipas, Mexico, a region that experienced a dengue fever epidemic in 2005. Pupal surveys were undertaken in 6 geographically stratified tire piles on each side of the border in August, September and October 2007 to determine: 1) presence/absence of disease vectors and 2) the impact of *Mesocyclops longisetus,* a predacious copepod naturally occurring in this region, against untreated control piles. *Toxorhynchites theobaldi*, a predacious mosquito, was found in tires at heavily vegetated sites in Brownsville but absent in Matamoros. Representative tires piles with *Tx. theobaldi* were considered an additional treatment category in the study design. The longitudinal effect of each treatment on total mosquito pupal counts was analyzed using a negative binomial population averaged Generalized Estimating Equation adjusted by environmental conditions such as vegetation and sun-exposure. Tires treated with *Mesocyclops longisetus* in Matamoros had significantly fewer mosquito pupae than control tires in both highly vegetated and sun-exposed sites. Tire piles with *Tx. theobaldi* significantly reduced the pupal populations of *Aedes* species. The effect of *Toxorhynchites theobaldi* control, however, significantly decreased over time as each tire in the *Toxorhynchites* group held more pupae in October than September. These results suggest that naturally occurring and introduced predator species can deliver significant reduction in *Aedes* immature populations. This may offer an important low cost and environmentally friendly alternative for *Aedes* control for resource-strapped local health departments on the border. Additional studies to better characterize the distribution of *Tx. theobaldi* on the US-Mexico border, and the longer term effectiveness of *Mesocyclops* would be valuable.

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COST-EFFECTIVENESS OF CHAGAS DISEASE VECTOR CONTROL STRATEGIES IN NORTHWESTERN ARGENTINA

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Control and prevention of Chagas disease rely mostly on residual spraying of insecticides. In Argentina, vector control shifted from a vertical to a fully horizontal strategy based on community participation in control and surveillance activities starting in 1992. The local effect of such strategy on Triatoma infestans, the main domestic vector, and on disease transmission has not been assessed. Based on official records from the Argentine Ministry of Health for the Moreno Department in the Province of Santiago del Estero (one of the poorest and most endemic districts in Argentina), we performed a cost-effectiveness (CE) analysis and compared the observed CE of the fully horizontal vector control strategy with the expected CE for a vertical strategy and a mixed strategy (i.e., vertical attack phase followed by horizontal surveillance). Total direct costs (in 2004 US$) of both horizontal and mixed strategies were 3.3 and 1.7 times lower than the costs of the vertical strategy, respectively, mostly as a consequence of reductions in personnel costs. The estimated CE ratios (measured in US$ per averted human case) for the vertical, mixed and horizontal strategies were 132, 78 and 45, respectively. When per diems were excluded from the costs (using a sensitivity analysis that simulated the decentralization of control activities), the CE of vertical, mixed and horizontal strategies was reduced to 60, 42 and 32 US$ per averted case, respectively. The mixed strategy would have averted between 1.6 and 4.0 times more human cases than the fully horizontal strategy, and even at a higher CE ratio, would have been the most cost-effective option to interrupt parasite transmission in Moreno. This is the first report of a CE analysis applied to compare different Chagas disease vector control strategies in Argentina, and the third in the Americas. Although underutilized for vector-borne diseases, CE analysis allows a better understanding of current strategies and improved planning of future interventions.

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THE CHAGAS VECTOR, *TRIATOMA DIMIDIATA*, SPECIES AND CRYPTIC SPECIES OCCUR IN SYMPATRY IN GUATEMALA AND MEXICO

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Chagas disease remains intractable in Mesoamerica due to the difficulty of controlling the most important Chagas vector in this region, *Triatoma dimidiata*; in part because of the enormous diversity of the vector across its geographic range. Recently, in isolates from: Mexico, Guatemala, Honduras, Nicaragua, Panama, Colombia and Ecuador, 31 haplotypes were identified falling in four distinct groups, including one that is proposed to be a separate species (*T*. sp. aff. *dimidiata*). This proposed cryptic species was found in Chiapas and Yucatan, Mexico; Peten, Guatemala; and Yoro, Honduras. Another study indicated that *T. dimidiata* and the distinct clade (*T*. sp. aff. *dimidiata*) both occur in the southern Yucatan peninsula state of Campeche, however, were associated with distinct geographic areas and habitats. We add data from many isolates from the geographic region that contains *T*. sp. aff. *dimidiata*, and others from Belize, El Salvador and Costa Rica. Using the nuclear ITS2 marker we identified two new haplotypes and show that the proposed new species is also found in Belize and occurs in sympatry with *T. dimidiata* in Yucatan, Mexico, and Peten, Guatemala. The large genetic distance between *T. dimidiata* and *T*. sp. aff. *dimidiata* shown by ITS2 is also supported by mitochondrial *CytB* sequence data.

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PREVALENCE OF *TRYPANOSOMA CRUZI* IN TRIATOMINE VECTORS IN THE SOUTHWESTERN UNITED STATES

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Approximately 10 million people in Latin America are infected with *Trypanosoma cruzi*, the causative agent of Chagas disease, which causes ~23,000 deaths annually. Although vector control strategies throughout Latin America have greatly reduced transmission of the parasite, Chagas disease continues to remain a major cause of morbidity and mortality

in many endemic countries. In the United States, Chagas disease exists almost exclusively in an active zoonotic cycle; the parasite is found in at least 17 mammal species. Chagas may be emerging as a human disease within the U.S. To date only six autochthonous cases of *T. cruzi* infection have been documented in the United States. We identified the most recent case in New Orleans, Louisiana in June 2006. However, 12 million Hispanic immigrants are thought to be in the United States, many coming from endemic areas, and possibly introducing new strains of *T. cruzi*. The AABB (formerly known as the American Association of Blood Banks) has confirmed 493 cases of *T. cruzi* infection from 1,667 repeat-reactive blood donations so far in 2008. Little current information exists on the triatomine vectors of *T. cruzi* within the U.S., and their abilities to transmit the parasite. We are determining the prevalence of *T. cruzi* infection in three of the most common species of triatomines found in the southwestern U.S.: *Triatoma rubida, T. recurva, and T. protracta*. Forty-three triatomines were collected in California and Arizona April-June 2007. By PCR, 22% of 23 *T. protracta* were infected with *T. cruzi*, with a higher prevalence in *T. protracta* from California. No *T. cruzi* was found in 19 *T. rubida* or the single *T. recurva* collected in Arizona. These results suggest that particular species of triatomines may be more likely to harbor the parasite than others.

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EVALUATION OF MULTI-DRUG THERAPY IN THE U.S.A. USING DAILY RIFAMPIN

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Hansen's Disease (leprosy) has been effectively treated by multi-drug therapy (MDT) with dapsone, clofazimine, and rifampin since 1982. Patients with multibacillary (MB) disease are at a higher risk for relapse following 2-year MDT including monthly rifampin, as recommended by the World Health Organization. The National Hansen's Disease Programs (NHDP) began a trial of MDT including daily rifampin in 1992. The occurrence of relapse after treatment was studied in patients treated and followed from 1988 - 1997, enabling a minimum of 10 years follow-up. A retrospective review of 351 medical records was performed for patients admitted from January 1988-December 1997. Patients receiving prior treatment were excluded, and 158 fulfilled the eligibility criteria. Data collected included demographic information, bacterial indices, treatment, and occurrence of leprosy reactions. MB patients comprised 77% of the cases reviewed. Overall, MB cases were treated with 2 or 3 drug protocols at rates of 36% and 35%, before and after 1992, respectively. The other 6% of the MB cases were treated with an alternative treatment protocol. Only one case of relapse was found within the study population, and this patient underwent 2-drug therapy versus 3-drug therapy. Although the patient population is small, these data are remarkable for the absence of relapse with daily rifampin, as contrasted with the WHO experience with monthly rifampin. Thus, the NHDP regimen with daily rifampin seems highly effective and may be superior to WHO regimens in avoiding relapse.

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PHASE I CLINICAL TRIAL OF V3526, VIROLOGICAL AND SEROLOGICAL ANALYSES

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Safety, tolerability, and immunogenicity of a candidate live, attenuated vaccine (V3526) for Venezuelan equine encephalitis (VEE) virus subtype IA/B was determined in a Phase I clinical trial. The study was a single-center 14-day in-patient investigation using different dose levels of vaccine in two cohorts. Volunteers were monitored for signs or symptoms of local or systemic adverse events (AEs), including fever, chills, headache, and neck stiffness. Blood samples and nasal/throat swabs were taken for evaluation of serum viremia, viral shedding, and serological responses. While no viremias were detected in any volunteer, positive throat swabs and/or nasal swabs from several of the 20 volunteers coincided with the period of febrile reaction. Of the seven isolates sequenced, five were identical to the consensus sequence of the vaccine. Two isolates, from both a throat washing and nasal swab of a single vaccine recipient, contained a single mutation in the E2 glycoprotein resulting in lysine to asparagine change at position 62 of the PE2 glycoprotein. Mutations at this position were not previously described for VEE or other alphaviruses. Based upon these results, the AEs encountered during the clinical trial are not due to reversion of the attenuated phenotype, but are an unanticipated consequence of the replication of the vaccine virus. Vaccination with a single dose of 125 PFU of V3526 elicited neutralizing antibodies in all 10 volunteers. In a second cohort that was vaccinated with 25 PFU, eight of 10 recipients developed neutralizing antibody responses. The neutralizing antibody responses were detectable over the entire 180-day follow-up period.

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SURVIVAL OF NEUTRALIZING ANTIBODY IN PREVIOUSLY RABIES VACCINATED SUBJECTS: A PROSPECTIVE STUDY SHOWING LONG LASTING IMMUNITY

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Many physicians encountering to potential rabies exposures and travel medicine are frequently asked how long previous pre- or post-exposure rabies vaccination induced immunity persists. A prospective study on one hundred and eighteen rabies vaccine recipients who had received pre- or post-exposure regimens with tissue culture rabies vaccines by intramuscular or intradermal schedules 5-21 years previously was conducted. Rabies neutralizing antibody was detectable in the sera of all subjects on day 0. They then received one intradermal 0.1 mL booster injection on days 0 and 3. Neutralizing antibody determination was carried out on days 5, 7 and 14. All except one subject showed an accelerated antibody response following the two booster injections. Vaccination with a WHO recognized tissue culture rabies vaccine evokes long lasting immunity. This study supports current recommendations that immunity is long lasting and that boosters without immunoglobulin are sufficient even when prior vaccination was longer than 5 years previously.

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EVALUATION OF THE IMPACT OF OVERSEAS PRE-DEPARTURE TREATMENT FOR INTESTINAL PARASITES AMONG MONTAGNARD REFUGEES MIGRATING FROM CAMBODIA TO NORTH CAROLINA, USA

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We evaluated the effectiveness of an overseas pre-departure regimen of 5 days of albendazole for presumptive treatment of intestinal parasites by examining stool specimens in treated and untreated Montagnard refugees after arrival in the US. Among 815 refugees evaluated, fully treated refugees had a significantly lower prevalence of helminths (11/777 [1.4%]), specifically hookworm and Ascaris lumbricoides, compared with untreated pregnant women (3/15 [20%]), (p<0.001). Multivariate analysis revealed that treatment was associated with significantly lower rates of infection with helminths but not protozoa. Post-arrival gastrointestinal symptoms were not associated with findings on stool examination. Our evaluation suggests that while additional studies are needed to determine optimal treatment regimens for intestinal parasites, especially among young children and pregnant women, a five-day course of pre-departure albendazole was effective in reducing helminthic infection in treated refugees

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THE CLINICAL PROFILE OF PATIENTS PRESENTING WITH DENGUE DURING THE OUTBREAK IN 2006 TO THE EMERGENCY DEPARTMENT OF AN URBAN TERTIARY CARE HOSPITAL IN INDIA

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This study was undertaken to determine the clinical profile of patients presenting with dengue during the outbreak in 2006 to the emergency ward of an urban tertiary care hospital. The current study was conducted in the Emergency Department at the All India Institute of Medical Sciences, New Delhi between August and October 2006. Patients presenting with short febrile illness and found to have thrombocytopenia were included in the study. Of 3707 cases included, 2834 (76.4%) were males, with similar distribution of parameters studied between both sexes. The mean (±SD) age was 25.51 (±12.83) years, mean hemoglobin level 12.5 (±3) g/dL, hematocrit 36.9 (±8.3), platelet count 50875/cmm (±22090) and total leukocyte count 6392/cmm (±3778). During the outbreak 15 patients died due to dengue hemorrhagic fever and shock. The mean age of patients who succumbed to the illness (33.6 ± 16.13) was higher than those who recovered (25.48 ± 12.8; p=0.072). The platelet counts of the patients who died were significantly lower (39571 ± 18923/cmm) than those who recovered (50918 ± 22093/cmm; p=0.043). Young males are noted to be more susceptible to probable dengue fever during the recent outbreak of dengue but the illness is more severe among older individuals. Lower platelet counts may preempt mortality.

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RETROSPECTIVE STUDY OF ANTIBIOTIC RESISTANCE AMONG *SALMONELLA ENTERICA* ISOLATES FROM 2005-2007

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During 2005 to 2007, we received 19165 blood cultures from the patients with clinically suspected enteric fever at Tribhuvan University Teaching Hospital, Kathmandu, Nepal. These isolates were identified and subjected for antibiotic susceptibility testing following CLSI standards. Approximately 7% of blood culture samples showed positive growth, of which 6% were *Salmonella enterica* serotypes *Typhi* and *Paratyphi-A.* One percentage of the isolates included *Staphylococcus aureus, E.coli, Klebsiella pneumoniae, Pseudomonas spp,* viridans streptococci*, Acinetobacter calcoaceticus, Citrobacter spp, Enterobacter spp and Enterococcus faecalis*. Among 1119 *Salmonella* isolates, about 6% were resistant to at least two classes of antibiotics (multidrug resistant or MDR). Almost equal percentages (about 3% each) of both *salmonella enterica* serotypes *Typhi and Paratyphi-A* isolates were found to be MDR. Many of the MDR *Salmonella*e were resistant to ampicillin, ciprofloxacin and co-trimoxazole. Among the MDR isolates, 95% were sensitive to ceftriaxone. The mechanism of multidrug resistance among these isolates of *S. enterica* needs to be explored further.

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THE CLINICAL PATTERN AND COMPLICATIONS OF SEVERE MALARIA IN PARTS OF THE IMO RIVER BASIN OF NIGERIA

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A three year retrospective study was carried out to determine the clinical pattern and complications of severe malaria in parts of the Imo River Basin of Nigeria. The medical records of all cases of malaria admitted to Aboh Mbaise general hospital, Imo state, Nigeria from 2004 to 2007 were analyzed. During the study period, 246 patients were diagnosed with malaria. The history of fever was generally present and in 78.8% of the cases it was associated with chills and rigor. Jaundice(23.5%) and splenomegally(26%) were the most common physical abnormalities. Severe anemia(Hb<50g/l) occurred in 17.1% of the patients. The occurrence of two or more complications was observed in 17.9% of the patients. The duration of hospitalization varied from four days to two months, an average of one week. Seven patients(2.8%) died during hospital admission. This study shows that severe malaria in malaria endemic areas can be associated with complications which can possibly lead to death. There is need for availability of adequate chemotherapeutic agents for early treatment of malaria and easy assessibility of health care facilities in these areas to avert complications and possible death arising from severe malaria.

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IDENTIFICATION OF IMUNODOMINANT REGIONS OF LEPTOSPIRAL IMMUNOGLOBULIN-LIKE PROTEINS FOR USE IN THE DIAGNOSIS OF LEPTOSPIROSIS

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There is an urgent need for improved diagnosis of leptospirosis, a life-threatening disease whose clinical presentation during early-phase illness is frequently confused with dengue and other causes of acute fever. We previously showed that infection in humans induces strong antibody responses to Leptospiral immunoglobulin-like (Lig) proteins A and B (MW 128 and 201kD, respectively). In this study we used a multi-antigen print

immunoassay to identify seroreactive fragments of Lig proteins, which in turn may be used for the development of a rapid serodiagnostic test. We expressed and purified eleven recombinant fragment (31kD-70kD) of LigA and LigB from *Leptospira interrogans* serovar Copenhageni. A LigB fragment (aa 582-947) was found to be the immunodominant fragment, recognized by acute-phase sera (mean 7.4±3.6 days of illness) from patients from an urban region of Brazil, where serovar Copenhageni is the agent for leptospirosis. IgM antibodies to this fragment were detected in 88% (46/52) of the leptospirosis patients. Among sera from control subjects from a high-risk slum community, 2% (1/40) demonstrated reactivity to this fragment. The sensitivity of the IgM response to the LigB aa582-947 fragment was 30% (6/20) when acute-phase sera (mean 4.7±1.9 days of illness) was evaluated from patients from rural Thailand, where transmission of leptospirosis is due to non-Copenhageni serovars. However, sensitivity increased to 75% when additional LigB fragments (aa 52-581 and 131-649) were incorporated in the assay and combined IgM and IgG responses were measured. Overall specificity of the assay was 97% when sera was evaluated from Thai control subjects. Together these findings suggest that a LigB-based serological test may be feasible approach to diagnosing leptospirosis in a range of epidemiological situations. Furthermore, the use of immunodominant fragments in rapid diagnostic formats may aid prompt and timely identification of leptospirosis which is required to reduce the high mortality associated with severe forms of the disease.

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OPHTHALMOMYIASIS BY CALLIPHORIDAE LARVAE IN A 16-YEAR-OLD FEMALE FROM HAWAII

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Our case is of a 16 year old female with no previous medical conditions. Two days before initial presentation, patient remembers being struck in the right eye by a fly. She presented to clinic with a right sided conjunctivitis. She was treated with erythromycin ointment and sent home. Patient did not respond to antibiotics are returned to clinic two days later. She stated that she could see "worms" travel through her field of vision. She was referred to Ophthalmology, who noted three larval entities on thorough examination. The larvae were excised and sent to entomology for evaluation. Final report was third instar larvae from the family Calliphoridae, species indeterminate. Patient recovered with no long term sequlae. Human myiasis is a rare infection in the United States, especially Hawaii, but may be increasing in incidence. Ophthalmomyiasis is the infection of ocular tissue by any species of flies. Ophthalmomyiasis accounts for 24% of all reported cases of myiasis. Ophthalmomyiasis externa is the infection of tissue surrounding the globe and is a more common form. Ophthalmomyiasis interna is the direct invasion of the globe by larvae, usually the anterior chamber. Most cases of ophthalmomyiasis in healthy tissue are by the family Ostridae, an obligate parasite. In contrast, Calliphoridae flies, also known as blowflies or screwworms, are facultative parasites which infect necrotic tissue or cadavers. Forensic entomologists use the growth staging of Calliphoridae larvae in estimation of time of death for legal cases. Calliphoridae rarely will infect healthy tissue, a fact which has been utilized in the practice of maggot debridement therapy. This is the first reported case of ophthalmomyiasis in previously healthy tissue by Calliphoridae larvae. Calliphoridae myiasis in the state of Hawaii has not been previously reported.

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PERCEPTIONS OF MOTHERS AND HOSPITAL STAFF OF PAEDIATRIC CARE IN 13 PUBLIC HOSPITALS IN NORTHERN TANZANIA

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User and provider perceptions of quality of care are likely to affect both use and provision of services. However, little is known about how health workers and mothers perceive the delivery of care in hospital paediatric wards in Africa. Paediatric staff and mothers of paediatric inpatients were interviewed to explore their opinions and experience of the admission process and conditions on the ward. Overcrowding, unsanitary conditions and lack of food were major concerns for mothers on the ward, who were deterred from seeking treatment earlier due to fears that hospital admission posed a significant risk of exposure to infection. While most staff were seen as being sympathetic and supportive to mothers, a minority were reported to be judgemental and authoritarian. Health workers identified lack of trained staff, overwork and low pay as major concerns. Staff shortages, lack of effective training and equipment are established problems but our findings also highlight a need for wards to become more parent-friendly, particularly with regard to food, hygiene and space. Training programmes focused on professional conduct and awareness of the problems that mothers face in seeking and receiving care may result in a more supportive and cooperative attitude between staff and mothers.

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HEALTH FACILITY-BASED ASSESSMENT OF THE BURDEN OF MALARIA IN LUANDA, ANGOLA

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As urbanization in Africa progresses, understanding the epidemiology of malaria in urban areas is needed for strategic allocation of resources to prevent and treat malaria. Luanda is the capital city of Angola, with greater than a third of the total population of the country, but little is known about the epidemiology of malaria in Luanda. In March 2008 (malaria transmission season), we conducted a health facility-based survey of patients with fever or history of fever to determine the proportion that had microscopy-confirmed malaria and to explore risk factors for malaria in Luanda. We selected 30 health facilities (HF) in Luanda and enrolled up to 30 patients in each with fever or history of fever. Each underwent blood film for malaria and answered a questionnaire. We enrolled 864 patients; 3.7% had microscopy-confirmed malaria. When stratified by distance of HF from city center, 1.4% of patients at HF < 15 km from city center had malaria, compared with 9.2% of patients at HF ≥ 15 km from city center. In multivariate analysis, age <5 years was most protective (OR 0.30, p-value 0.004), and presenting to a HF ≥ 15 km from city center (OR 6.69, p-value <0.0001) was most associated with risk of having malaria. Only 15.6% of patients with malaria had traveled outside Luanda, and though there was a trend toward more malaria in travelers, this was not statistically significant. The prevalence of microscopy-confirmed malaria in febrile patients in urban Luanda is very low, but risk of malaria increases with increasing distance from the urban center. Our results underscore the need for resources for prevention, diagnosis, and treatment of malaria in rural areas surrounding Luanda. In urban areas, improved differential diagnosis of fever by health care workers and improved laboratory diagnosis for malaria are vital.

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IN-VIVO EFFICACY OF AMODIAQUINE-ARTESUNATE FOR TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN CHILDREN IN WESTERN KENYA

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Artemisinin-based combination therapy (ACT) is now first line treatment for uncomplicated *Plasmodium falciparum* malaria in most of sub-Saharan Africa, but questions regarding the appropriate partner drug to protect against development of artemisinin resistance persist. Though amodiaquine efficacy generally remains higher than chloroquine efficacy, given the cross-resistance between chloroquine and amodiaquine, there are concerns regarding the efficacy of amodiaquine-artesunate in areas of high chloroquine resistance. We conducted a 28 day *in-vivo* efficacy trial of amodiaquine-artesunate in 103 children ages 6-59 months in western Kenya with smear-confirmed uncomplicated *P. falciparum* malaria. The 28-day uncorrected adequate clinical and parasitological response (ACPR) was 69.0%, with 15.5% Late Clinical Failure and 15.5% Late Parasitologic Failure rates. The PCR-corrected 28 day ACPR was 90.2%. Clinical risk factors for recurrent infection (recrudescences and reinfections) were lack of documented fever at enrollment and low weight-for-age Z-score. The presence of single nucleotide polymorphisms *pfcrt* 76T and *pfmdr-1* 86Y at baseline was associated with increased risk of recurrent infections, both reinfections and recrudescences, but there was not a significant increase in frequency of these alleles on the day of failure compared to baseline in children with recurrent infections. Although artemether-lumefantrine is the first line ACT in Kenya, amodiaquine/artesunate is registered in Kenya as an option for treatment of uncomplicated *P. falciparum* and remains an effective alternative to artemether-lumefantrine in western Kenya. Continued amodiaquine monotherapy in the private sector may jeopardize the future use of amodiaquine-artesunate as an alternative artemisinin-based combination therapy.

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TREATMENT OF SEVERE SEPSIS WITH ARTEMETHER-LUMEFANTRINE IS ASSOCIATED WITH DECREASED MORTALITY IN UGANDAN PATIENTS WITHOUT MALARIA

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Artemisinins have anti-inflammatory properties and improve survival in murine sepsis models. We enrolled 382 patients with severe sepsis at two hospitals in Uganda and analyzed the association between the use of artemether-lumefantrince (A-L) and combined in-hospital and 30 day mortality in patients with or without malaria. In patients with negative malaria smears (n = 328 of 379), Kaplan-Meier curves revealed decreased combined inpatient and 30 day mortality among patients receiving A-L vs. those who did not (20.6%, s.e. = 10.6 vs. 48.8%, s.e. = 3.2; Log rank $\chi^2 = 3.93$, $p = 0.048$). The decrease in mortality associated with A-L was maintained in the most clinically ill patients determined by Karnofsky Performance Scores ≤ 50 (16.7%, s.e. = 15.2 vs. 58.2%, s.e. = 3.7; Log rank χ^2 3.94, $p = 0.041$). Further investigation into the properties of A-L is needed to maximize the treatment of sepsis without compromising malarial susceptibility.

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A SURVEY OF PHLEBOTOMINE SAND FLIES IN THREE FOCI OF CUTANEOUS LEISHMANIASIS IN THE HO DISTRICT OF THE VOLTA REGION OF GHANA

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Sand flies are the proven vectors of leishmaniasis worldwide. Although the disease is rare in West Africa; there have been sporadic incidences of cases in West African countries, including Ghana. First-time definitive evidence of human cutaneous leishmaniasis (CL) from *L. major* were dreported previously. These infections were in the form of PCR testing and DNA sequence analysis of skin sample biopsies from residents in endemic areas. The Ghana Health Service Annual Report of 2003, presented data of 8,876 cases in the south eastern part of the country from the Districts of Ho (8,533), Hohoe (176) and Kpando (167). Entomological surveillance studies of sand fly species in 3 foci of CL were undertaken in 2005 to identify potential vectors of the disease in the region. A total of 1,645 phlebotomine sand flies (Diptera: Psychodidae) were collected using sticky paper traps. A total of 19 *Sergentomyia* species were captured and identified. *S. africana africana* (25.84%), *S. squamiplueris* (25.78%) and S. ghesquierei (17.26%) were the most abundant species collected, whereas *Phlebotomus* spp. were not collected at all using this surveillance method. Sand fly populations peaked in July, with 676 (41.09%) of all flies captured during this month. The lowest sand fly activity occurred in February, with only 9.48% (156) captured. The absence of Phlebotomus is peculiar given the apparently high leishmaniasis transmission rates that have occurred in the past in these areas. Historical records also indicate a paucity of human CL vector(s) present in this region, suggesting that *Sergentomyia* spp. may play a significant role in the transmission of CL. Further studies are warranted to investigate this possibility in this region of Ghana.

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HIV, GRAVIDITY AND MALARIA DURING PREGNANCY IN SOUTHERN MALAWI: ANTAGONISTIC INTERACTION?

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The purpose of this analysis was to determine the effect of HIV and gravidity on parasitemia during pregnancy among women in Malawi. Between 2005 and 2006, we collected data on HIV seropositivity, parasitemia, and sociodemographic characteristics from a cohort of pregnant women at the Mpemba and Madziabango clinics in Blantyre District, Malawi. A total of 1,496 women were enrolled in the study. HIV seroprevalence was 13.5%. Over the follow-up period, 46.5% of the women became parasitemic. Multivariate analysis revealed antagonistic effect measure modification (EMM) by gravidity for the association between HIV and parasitemia. Among primigravid women, the risk of parasitemia among HIV-infected women was 0.1 (95%CI: 0.01, 0.6) times the risk among HIV-uninfected women. However, among multigravid women, the risk of parasitemia among HIV-infected women was 2.5 (95%CI: 1.2, 5.0) times the risk among HIV-uninfected. Examining sociodemographic variables among primigravid women, HIV-infected women were 3.6 (95%CI: 1.4, 8.9) times as likely to report always or usual bed net use, 6.0 (95%CI: 1.9-18.4) times as likely to report receiving an SP dose prior to enrollment, and 5.5 (95%CI: 2.3, 13.2) times as likely to have secondary education compared to HIV-uninfected women. Furthermore, HIV-infected primigravidae were older (t = 2.25, p = 0.02) and weighed more on average (t= 2.66, p=0.01), than HIV-uninfected primigravidae. However, after controlling for sociodemographic variables,

antagonistic EMM persisted. In this cohort, HIV infection was found to be associated with an increased risk of parasitemia over the follow-up period among multigravidae, but a decreased risk among primigravidae. Although, HIV-infected primigravidae had higher education levels and were more likely to practice malaria prevention behaviors, controlling for these characteristics failed to remove or attenuate the observed antagonistic EMM. However, the strong observed differences in preventive behaviors suggests that in this population, HIV-positive primigravidae may have a complex of malaria protective factors that is counteracting the adverse effect of HIV.

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NOVEL RECOMBINANT ANTIGEN-BASED ELISA FOR ASSESSING EXPOSURE TO MALARIA IN AT-RISK BLOOD DONORS

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Using a cocktail of recombinant antigens, an antibody detection ELISA system was developed for assessing exposure to malaria in donor blood samples. Initially samples derived from proven *Plamodium falciparum, P. vivax, P. malariae* and *P. ovale* infection were used as indicators of species level reactivity. The assay system was optimised by testing specific parameters such as sample dilution, appropriate anti-human IgG conjugates and different assay timings. This test system was further developed into a commercial format and evaluated in blood banks located both in endemic and non-endemic regions. Based on the data generated, the assay was found to be a specific and reliable method for assessing exposure of donors to malaria infection due to either single or mixed infections and may prove useful as an evaluation tool for re-utilization of blood samples deferred on the basis of travel history.

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COMPLIANCE TO UNSUPERVISED ACTS IN YOUNG BENINESE CHILDREN: DATA FROM A RANDOMISED TRIAL COMPARING ARTEMETHER-LUMEFANTRINE TO AMODIAQUINE-ARTESUNATE FIXED-DOSE FORMULATIONS

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In the setting of a randomised open trial comparing unsupervised artemether-lumefantrine to unsupervised amodiaquine-artesunate fixed-dose formulation (96 children in each arm), we assessed compliance to both regimens. On day 3 (i.e. 12 hours after the last dose of AL, and 24 hours after the last dose of ASAQ), informations on compliance to the drug regimen were collected at the patient's home, study drugs blisters were collected at home, and a small amount of blood was taken in all patients for whole blood drug level analysis. Data on day 3 levels were also obtained in 60 children from the same area who were given supervised treatments, 30 of them having received AL (with fat), and 30 ASAQ. Blisters' recovery at home was fruitful in 81 (84.4%) children who were given AL and 89 (93.7%) children who were given ASAQ. Compliance was declared suboptimal in 16 children given AL and in 9 given ASAQ. Overall, among the 171 children who had received an ACT and were included in the evaluability analysis, 155 (75 AL and 80 ASAQ) had declared having taken at home a total number of doses equal to the total prescribed doses. When the effectiveness analysis was restricted to these 155 children, day 28 PCR-corrected success rates were 94.7% with AL and 92.5% with ASAQ (94.0% with AL and 93.2% with ASAQ in the full set of evaluable patients) Mean drug levels for lumefantrine were 1.31±0.87 µg/mL and 0.97±0.7 µg/mL in the supervised (28 children) and unsupervised (82 children) groups, respectively (p=0.03). Mean levels of monodesethylamodiaquine (mdAQ) were 0.31±0.08 µg/mL and 0.28±0.16 µg/mL in the supervised (30 children) and unsupervised (91 children) groups, respectively (p=0.05). Mean lumefantrine and mdAQ values did not differ between success and failure outcomes, nor between presence and absence of new infection during the follow-up. Suboptimal compliance with AL and ASAQ was not rare, but did not reduce effectiveness. Lack of concomitant ingestion of fat with AL may partly explain marked decreased PK lumefantrine values observed with unsupervised AL treatment.

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CLIMATE CHANGE AND TROPICAL PUBLIC HEALTH

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Climate change has direct and indirect consequences for human health. Heat waves affect health directly and are projected to take an increasing toll in developed and developing nations. Climate and extreme weather affect the range and distribution of vector-borne diseases, while warming and extremes also encourage pests and pathogens afflicting forests, crops, livestock, wildlife and coral reefs. Several issues underlie the observed climate- and weather-related changes in disease distribution: 1. Since 1950, nighttime and winter warming have occurred twice as fast as has overall global warming; 2. The pace of warming in temperate, boreal and polar latitudes is occurring faster than warming in the tropics; and 3. Since 1957, the first International Geophysical Year, when many global measurements were initiated, the world ocean has accumulated twenty-two times the amount of heat as has the atmosphere, accelerating the global hydrological cycle. Extremes are particularly conducive to upsurges of pest populations, e.g., mosquitoes and rodents. Drought in East Africa, in association with a warming Indian Ocean, led to the explosive outbreak of Chikungunya fever in 2004-06, which has since spread to Italy. Sahel drought is related to upsurges of meningococcal meningitis and, in Latin America, heavy rains and flooding have been associated with outbreaks of dengue fever. Sequences of extremes create conditions conducive to "clusters" of mosquito-, rodent- and water-borne disease outbreaks. Excess carbon dioxide itself (CO_2) has consequences for human health. Ragweed grown under elevated CO_2 levels produces pollen disproportionately to increases in its stem growth, and the pollen proteins become more allergenic. Additionally pollen grains are food for the larvae of anopheline mosquitoes, thus CO_2 fertilization may contribute to increasing malaria. On the other hand, clean energy solutions can improve health directly and provide the basis for development, clean water, food supplies, education, health facilities, and small-scale development - that form the basis for health in developing tropical nations.

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ENHANCED EFFICACY OF AMODIAQUINE AND CHLORPHENIRAMINE COMBINATION OVER AMODIAQUINE ALONE IN THE TREATMENT OF ACUTE UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN CHILDREN

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This study was undertaken to evaluate the comparative efficacy of amodiaquine (AMQ) alone and the combination of AMQ and chlorpheniramine (CP) in the treatment of acute uncomplicated malaria in children. Of the 110 children enrolled in the study, 103 with acute uncomplicated malaria, aged 6 months to 12 years, were evaluated using the 14-day modification of the WHO field test. The patients were randomized to 2 groups. Group 1 received supervised treatment with AMQ alone (10 mg AMQ base/kg daily for 3 days), while group 2 received supervised treatment with AMQ (same dose as group 1) plus CP (AMQCP) for 7 days. Both treatment regimens were well tolerated and no patient was withdrawn as a result of recurrent vomiting or drug-related

adverse events. There was no significant difference in mean fever and parasite clearance times. The cure rates at day 7 were 90.2 versus 100% (ρ = 0.027) for AMQ versus AMQCP, while the day 14 cure rates were 85.9 versus 98.1% for AMQ versus AMQCP, respectively (ρ= 0.016). In conclusion, the combination of AMQ plus CP proved significantly more effective than AMQ alone in the treatment of acute uncomplicated falciparum malaria, most probably due to the enhancement of the antimalarial effect of AMQ by CP. The combination of AMQCP could be a better alternative to AMQ alone as a companion drug in artemisinin-based combination therapies.

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CHANGING TRENDS OF DENGUE EPIDEMIC DURING RECENT OUTBREAK IN NORTH INDIA

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This study was undertaken to investigate the changing trends in clinical profile, effect on liver function and risk factors for bleeding in adult patients during the dengue outbreak in North India. This prospective observational study was conducted in the emergency department of an academic urban hospital during 2004 outbreak in New Delhi, India. All consecutive adult patients with clinical features satisfying the WHO criteria for Dengue fever were recruited in the study. Patients underwent detailed clinical evaluation including laboratory and radiological investigations towards symptoms of dengue. Positivity for "IgM only" was considered as primary infection and presence of "both IgM and IgG" was considered the indicator of secondary infection.Statistical analysis was done using SPSS version 16. Out of 265 eligible patients screened, 208 cases were included in the study. Mean age of patients was 27.1 years (range 12-80 years); 78% were males. The cases were defined as DF in 122, and DHF/DSS in 86; 176 were primary and 32 secondary infections. The predominant presentations were fever(100%),myalgia (61.5%), vomiting(42%), headache(36%), rash(24.3%), abdominal pain(16.8%), seizures (1.6%),and bleeding manifestations 103(49.5%). Myalgia Odds Ratio (OR) =3.13 p<0.001, hepatomegaly OR=2.56 p=0.036, morbiliform rash OR=2.53 p=0.004 and headache OR=1.94 p=0.024, were independently associated with increased likelihood of spontaneous bleeding. The prominent unusual feature observed was moderate elevation of transaminases: in DF and DHF/DSS; the mean (range) AST [125(23-448),137(24-609)] IU (p=0.3), ALT [111 (42-325), 115(32-461)] IU (p=0.6) serum bilirubin [0.76 (0.3-1.8), 0.8 (0.3-1.8] mg/dl (p=0.1), and serum alkaline phosphatase[174(59-530), 153(56-610)] IU (p=0.3) All patients recovered except one (0.48%) who succumbed due to intracranial hemorrhage. Fever, myalgia, rash, thrombocytopenia, increasing age and decreased mortality rate were the predominant manifestations of this outbreak. Asymptomatic elevation of transaminases along with normal serum bilirubin and alkaline phosphatase were observed independent of severity of infection. The study corroborates trends in dengue outbreaks reported from other parts of Asia. Collaborative studies integrating different centres in Asia are needed for better understanding the regional variations of this infection

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ACTIVITY OF AQUEOUS METHANOL AND WATER EXTRACTS OF *OSYRIS LANCEOLATA* ON ATCC 2592223 *STAPHYLOCOCUSS AUREUS* AND CLINICAL ISOLATES OF *STAPHYLOCOCUSS AUREUS*

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Osyris lanceolata, an evergreen shrub in the family *Santalacea*, is found in the drylands of Kenya. It is used to treat gastrointestinal and dermatological disorders in the local areas of Kenya (e.g. Kitui and Kajiado). The objective of this work was to investigate the anti-microbial activity of hexane, dichloromethane, aqueous methanol and water extracts from the stem, roots and stem bark of the plant, to explore possible scientific rationale for its use in folklore treatment of diseases. The extracts were obtained using sequential extraction technique from dried and ground plant samples. Anti-microbial activity of these extracts were evaluated against five bacteria and three fungi, which included ATCC 2592223 *Staphylococcus aureus*, ATCC 25922 *Escherichia coli*, ATCC 90028 *Candida albicans*, and clinical isolates of *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeroginosa*, *Microsporum gypseum,* and *Cryptococcus neoformanas*, obtained from KEMRI-Center of Microbiology Research. Disc diffusion technique was used to carry out the bioassays and the results indicated that the aqueous methanol and water extracts exhibited activity against standard strains of the gram-positive bacteria and the clinical isolates of *Staphylococcus aureus* at a concentration of 234 µg/ml and there was mininimal variation on inhibition of the micro-organisms which was not significantly different (p>0.05). The minimum inhibitory concentrations (MIC) ranged between 117 -234 µg/ml respectively. The study provided scientific proof of antimicrobial activity of *Osyris lanceolata* as used in ethnobotanical practice. This information is useful for further studies on the plant, specifically its constituents and their pharmacological activities.

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LABORATORY PROFILE OF DENGUE OUTBREAK IN A DEVELOPING NATION. (2006)

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This study was undertaken to correlate the clinical and laboratory profile of patients presenting with dengue during the outbreak in 2006 to an urban tertiary care hospital in a developing nation. This prospective observational study was conducted in the emergency department of an academic urban hospital between August and October 2006. All consecutive adult patients (12 years and above) with clinical features satisfying the WHO criteria for dengue fever were recruited in the study. Those who had fever duration of more than 7 days at presentation, other identified cause of fever or primary hematological illnesses such as aplastic anemia were excluded from the study. Patients underwent detailed clinical evaluation including laboratory and radiological investigations towards symptoms of dengue. Statistical analysis was done using SPSS version 16. Of 3707 cases included, 2834 (76.4%) were males.No significant difference in clinical parameters was determined based on gender. The mean (±SD) age was 25.51 (±12.83) years, mean hemoglobin level 12.5 (±3) g/dL, hematocrit 36.9 (±8.3), platelet count 50875/cmm (±22090) and total leukocyte count 6392/cmm (±3778). During the outbreak 15 patients died due to dengue hemorrhagic fever and dengue shock syndrome. 11 patients succumbed to intra cranial bleeding while in 4 patients hematemesis was identified as the cause of mortality. The mean age of patients who succumbed to the illness (33.6 ± 16.13) was higher than those who recovered (25.48 ± 12.8; p=0.072). The platelet counts of the patients

who died were significantly lower (39571 ± 18923/cmm) than those who recovered (50918 ± 22093/cmm; p=0.043). Young males were noted to be more susceptible to probable dengue fever during the recent outbreak of dengue in parts of northern India, but the illness was more severe among older individuals. Lower platelet counts may preempt mortality. Sero epidemiological studies in conjunction with the suitably directed public health initiatives- vector control, education among the masses are required for containing the burden of dengue in the developing world.

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FEASIBILITY, ACCEPTABILITY AND SAFETY OF USING ACTS IN HOME BASED MANAGEMENT OF FEVER IN RURAL UGANDA

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Uganda has been implementing Home-based Management of Fever (HBMF) since 2001. Due to the rising malaria parasite resistance to pre-packaged SP and CQ (Homapak) the health ministry changed to Artemisinin-based Combination Therapies (ACTs). The policy change recommended Athemether Lumefantrine (AL) as the first line for uncomplicated malaria. No prior experience had documented the feasibility, acceptability, and safety of using AL under HBMF thus a study was commissioned between June 2006- November 2007 in Iganga and Bugiri districts. The overall objective was to assess whether AL could feasibly be used under HBMF. Specific objectives were: to establish; acceptability and adherence of caregivers and community medicine distributors (CMDs), adverse effects and stability and quality of AL stored in homes. Qualitative and quantitative methodologies were used during the study. Drug stability tests were carried out on AL kept in six randomly selected villages and the health facility stores. Selected homes included grass thatched and iron roofed houses. Three tests at month 0, 3 and 6 were administered on a batch of collected tablets stored at each selected home at the national laboratory. Promptness to seeking treatment rose from 53% to 89%, adherence of caregivers to the correct treatment schedule rose from 79% to 93%, AL was acceptable to 97% caregivers and 3.5 % of caregivers reported minor side effects. Stability results for AL stored for up to six months at room temperature showed that the drugs were stable. All results complied with the manufacturer's end of shelf-life specifications for artemether and lumefantrine. There were no significant changes in the dissolution tests for lumefantrine. AL was highly acceptable to study communities. Most CMDs and caregivers adhered to prescription and treatment instructions. No serious adverse events were reported after AL use at community level. AL was stable under storage conditions in communities for a period of at least six months.

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EVALUATION OF HOME-BASED MANAGEMENT OF FEVER WITH ARTEMETHER-LUMEFANTRINE IN URBAN UGANDAN CHILDREN: A RANDOMIZED TRIAL

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Home-based management of fever (HBMF), promoting presumptive treatment of febrile children with pre-packaged antimalarials, has been advocated to ensure prompt effective treatment of malaria. However, there are potential downsides to HBMF, and no data on the health impact of HBMF using artemisinin-based combination therapies are available. We evaluated the impact of home delivery of artemether-lumefantrine (AL) for management of febrile illnesses in children on the incidence of antimalarial treatment and other clinical measures, as compared to the current standard of care in Kampala, Uganda. HBMF households were trained and given AL to keep at home for presumptive treatment of fever in participating children. Of 437 children randomized, 225 to HBMF and 212 to standard care, 365 (84%) completed 12 months of study follow-up. Significantly more febrile episodes were treated promptly with an effective antimalarial in the HBMF arm than in the standard care arm (58% vs. 8%, respectively, RR 7·18, 95% CI 4·58 - 11·27, p<0·0001). Comparing clinical outcomes at completion of the study, the proportion of participants in the HBMF arm with a positive thick blood smear was lower than in the standard care arm (2% vs. 10%, RR 0·21, 95% CI 0·07 - 0·64, p=0·006), but no other clinical differences were seen. Compared to the standard care group, the incidence of hospitalizations was lower in the HBMF arm (0·23 vs. 0·13, IRR 0·55, 95% CI: 0·31 - 0·99, p=0·047). The HBMF group received nearly twice the number of antimalarial treatments as the standard care group (4·66 vs. 2·53, IRR 1·72, 95% CI: 1·43 - 2·06, p<0·0001), and approximately five times the number administered for microscopically-confirmed cases of malaria in a comparable cohort of children (4·66 vs. 1·03, IRR 5·12, 95% CI: 4·24 - 6·35, p<0·0001). In this urban setting, HBMF with home delivery of AL substantially improved prompt treatment of fever with effective antimalarials, but had little impact on clinical outcomes. The lower incidence of hospitalization observed in the HBMF arm suggests a clinical benefit, but at the cost of substantial over-treatment with antimalarials.

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ELEVATED CEREBROSPINAL FLUID LEVELS OF NITRIC OXIDE ARE ASSOCIATED WITH PROTECTION FROM LONG-TERM COGNITIVE IMPAIRMENT IN CHILDREN WITH CEREBRAL MALARIA

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Low plasma levels of nitric oxide (NO) are seen in cerebral malaria (CM) but the relationship of systemic and central nervous system NO levels to long-term neurologic and cognitive impairment in CM are unknown. Serum levels of nitrate and nitrite (NOx) were measured in Ugandan children 3 to 12 years of age with CM (n=76), uncomplicated malaria (UM, n=68), and healthy community controls (CC, n=49). Cerebrospinal fluid (CSF) levels of NOx were measured in children with CM and 8 control children. Children 5 to 12 years of age were assessed for neurologic and cognitive impairment at enrollment/ discharge and 6 and 24 months later. Children with CM or UM had lower serum levels of NOx (adjusted for renal impairment) than CC (P=0.002 and P=0.02, respectively). Adjusted NOx levels did not differ significantly between children with CM and UM (P=0.54). Children with CM had higher CSF NOx levels than control children (P=0.05). Children with CM who had persistent cognitive impairment at both 6 and 24 months had lower levels of CSF NOx than those without impairment at both time points (P=0.06). In conclusion, in children with CM, serum levels of NO are decreased, but CSF NO levels are increased and elevated CSF NO is associated with long-term neuroprotection.

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AN ASSAY SYSTEM FOR QUANTIFYING HISTIDINE RICH PROTEINS IN HUMAN MALARIA BLOOD SAMPLES

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Histidine rich (HRP2 and other isoforms) proteins are shown to be specific indicators of *Plasmodium falciparum* infection in human blood samples. Currently only qualitative assays (rapid tests as well as ELISA) have been described. A reference homogeneous and purified histidine rich protein

is needed to quantify the binding kinetics in the enzyme immuno-assays (EIA). A sensitive HRP- capture ELISA system has been developed at Cellabs Pty Ltd by using monoclonal antibodies in a sandwich EIA format. Further by using a homogeneous and purified recombinant HRP-2 protein, a standard graph was developed in the capture ELISA system which formed the basis for quantifying the binding of histidine rich proteins. This has been verified by using the human blood samples containing varied burden of *P. falciparum* parasites that have assessed by microscopy. Data will be presented on the interrelationship between the parasite burden and the quantified HRP level as determined in the ELISA. A quantitative HRP-2 ELISA system may prove useful in analysis of malaria infection, in drug sentivity and vaccine studies.

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DETECTION OF SURFACE COATED SPECIFIC ANTIBODY OF *CANDIDA TROPICALIS* ISOLATES FROM CLINICAL MATERIALS BY ELISA TEST

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Candidiasis is a common opportunistic infection in immunocompromised hosts and candidaemia cause significant morbidity and mortality in this group of patients. Currently, *Candida tropicalis* infection is more common in countries like India. During *Candida* infection, significant amount of antibody is formed in the blood which may persist on the surface of *Candida* species in primary cultures. Thus there is a scope to exploit it by developing an ELISA kit to differentiate pathogenic and non pathogenic *Candida* species isolated from samples like sputum and urine, where there are chances of contamination. We developed an indirect ELISA kit for this study. Cells of *Candida tropicalis* (ATCC-750) were suspended in sterile water and added to ZR Bashing Bead Lysis Tube. Following this, the cells were lysed by agitation in vortex for 5 minutes and then centrifuged at 10,000 g for 1 minute. The supernatant was kept in boiling water bath for 5 minutes to stop all enzymatic activities. This was then mixed with coating buffer at different concentrations and used to coat the blank microwells. The wells were incubated at 37ºC for 3 hours and then kept overnight at 4ºC. After washing the plate 3-4 times, the culture supernatant was added, which was prepared by mixing primary culture isolates of *Candida tropicalis* from sputum and urine with diluting buffer. Further procedures of routine indirect ELISA tests were performed with optimized enzyme-conjugate and substrate-chromogen solutions. Thus, the washed primary culture supernatant was tested for the presence of specific antibody. In this experiment it was found that when microwell coating was done with 20 µg/mL concentration of the antigen extract, the maximum absorbance was obtained while absorbance of the negative controls were negligible. Positive results of culture supernatants correlated well with the clinical findings. In conclusion, in ELISA test to detect presence of specific coated antibodies in primary cultures of *Candida* from infected persons has been developed and standardized. This will help to differentiate pathogenic *Candida* species from contaminated *Candida* species present in clinical materials.

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INCREASING PREVALENCE OF NOSOCOMIAL FLUCONAZOLE RESISTANT *CANDIDA TROPICALIS* INFECTION IN KOLKATA, INDIA

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Worldwide, the most common opportunistic infection is caused by *Candida* spp. Among different *Candida* spp. currently *C. tropicalis* is most frequently isolated from clinical materials from South East Asian countries. Available reports from these countries also indicate their high fluconazole resistance. In India, particularly from Eastern part of India, reports on *Candida* spp.is mainly limited to individual cases.Considering these aspects, we were interested to study *Candida* spp. and their antifungal sensitivity patterns during last 5 years in a referral hospital of Kolkata, India. Clinical materials obtained from patients with suspected nosocomial fungal infections were inoculated on two sets of Sabouraud dextrose agar media and kept up to 21 days in incubator at 37ºC and 25ºC. *Candida* spp. were mainly isolated within 48 hours of incubation and they were identified by routine procedures: germ tube test, Chromagar study, colony morphology on CMA media, carbohydrate assimilation and fermentation tests. It was found that in 2003 and 2004, there was no *C. tropicalis* isolate among 28 *Candida* spp. isolated from different clinical materials which were mostly *C.albicans* (93%) and sensitive to fluconazole.However, the scenario was changed from 2005 and during 2005 - 2007, the number of *C. tropicalis* isolates were gradually increased from 45% out of 20 isolates in 2005, 47% out of 17 isolates in 2006 to 50% out of 20 isolates in 2007.Although all the isolates were sensitive to amphotericin B but most of them (84%), were resistant to fluconazole. Sensitivity pattern of *C. tropicalis* isolates to other tested antifungal drugs were almost same to *C. albicans*. At present, *C. tropicalis* is the most common isolate in nosocomial candidiasis patients in Kolkata and most of them are fluconazole resistant.The changing pattern of *C. albicans* and *C. tropicalis* and their altered antifungal resistance pattern is very important from epidemiological point of view in India.

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EFFECT OF AGRICULTURAL ACTIVITIES ON PREVALENCE RATES, CLINICAL AND PRESUMPTIVE MALARIA EPISODES IN CENTRAL CÔTE D'IVOIRE

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Agricultural activities, among other factors, can influence the transmission of malaria. In two villages of central Côte d'Ivoire (Tiémélékro and Zatta) with distinctively different eco-agricultural characteristics, we assessed *Plasmodium* prevalence rates, fever and clinically-confirmed malaria episodes among 171, 598, 714 and 245, 689, 795 children aged ≤ 15 years by carrying out repeated cross-sectional surveys, in Zatta and Tiemelekro, respectively. Additionally, presumptive malaria cases were monitored in health centres for a 3-year period. For quality control, 10% of the slides were randomly selected and re-examined by a second senior technician. In Tiémélékro, we observed a decrease in malaria prevalence rates from 2002 (86.1%) to 2005 (60.4%), which might be explained by changes in agricultural activities from subsistence farming to cash crop production. In Zatta, where an irrigated rice perimeter is located in close proximity to human habitations, malaria prevalence rates in 2003 (58.4%) were significantly lower than in 2002 (85.4%) and 2005 (66.0%), which coincided with the interruption of irrigated rice farming in 2003/2004. Although malaria transmission differed by an order of magnitude in the two villages in 2003, there was no statistically significant difference between the proportions of severe malaria episodes. In Zatta, there was a highly significant difference between the proportions of clinical malaria cases among children ≤ 15 years recorded in 2003 (8.5%) and 2005 (16.8%) (χ^2 = 19.67; df =1; P <0.001). In Tiémélékro, on the other hand, the proportion of diagnosed clinical malaria cases recorded in 2003 (16.5%) and 2005 (16.9%) was similar (χ^2 = 0.05; df =1; P = 0.823). Our study underscores the complex relationship between malaria transmission, prevalence rate and the dynamics of malaria episodes. A better understanding of this relationship can facilitate the starting of a control strategy.

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NOVEL ANTIHAEMORRAGIC EFFECT OF A NEW LOTION

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Secondary bacterial infection from accidental cuts, wounds and bruises a common health problem in developing countries with low standard of hygiene and inadequate first aid treatment. The purpose of this study was to determine the efficacy of a novel lotion containing oronsfranklo in stopping bleeding from accidental cuts, wounds and abrasions and healing of such accidental cuts. 150 adults comprising 80 males and 70 females presenting at the Accident Unit of University of Benin Teaching Hospital in Benin City were randomly recruited into the study. Strict confidentiality and Ethics committee approval of the Teaching Hospital was obtained before the commencement of study. Wounds and cuts sustained by the subjects were treated with the lotion using cotton wool buds. The composition of the lotion is as follows: salicylic acid 4 grams, ethyl acohol,100mls., and glycerine 3 mls. The salicylate was dissolved in the alcohol, glycerine was added and the mixture was allowed to stand. The bleeding from the cuts and wounds stopped immediately and wounds were completely healed by the third day after application of the lotion.

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A COMPARISON OF MICROSCOPY WITH RAPID DIAGNOSTIC TESTS FOR MALARIA IN RURAL GHANA

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Over-diagnosis of malaria threatens the sustainability of ACT deployment in Ghana. Rapid diagnostic tests (RDTs) to direct ACT use could be cost-effective but only if tests guide clinician prescribing. Data from East Africa suggest that RDTs have little impact on clinician behavior; however, this may not be the case in West Africa. Clinician diagnosis may also differ in areas where there is little or no access to microscopy. A individually randomized trial was conducted to compare the efficacy of microscopic diagnosis with clinical diagnosis on the impact of introducing RDTs on the appropriate prescription of anti-malarials. This study was conducted in 4 health facilities in Dangme West, southern Ghana, a rural district. One health facility has microscopy; while diagnosis in the others is purely clinical. Malaria accounts for 49% of reported outpatient cases. Following a baseline survey, febrile patients were randomly allocated to microscopy or RDT in the microscopy setting and to clinical diagnosis or RDT elsewhere. A blood film was taken for all patients. Sample size was 3500 and 3000 patients for microscopy and clinical sites, respectively. Day 28 follow-up is carried out for all study subjects. The primary outcome is the proportion of RDT test-negative patients prescribed an anti-malarial in both settings with several secondary outcomes. Preliminary results: 542 out of 602 patients presenting in the baseline period complained of fever or history of fever; 128 (23.6%) of these had fever on examination. 18 (13.2%) with fever and 73 (15.7%) without fever had a lab test for malaria parasites. Of these 17/18 with fever had a positive result compared to 42/73 with no fever. Of 33, 11 (33%) patients with a negative microscopy result still received an anti-malarial. 13 (39.4%) received an antibiotic and 3 received both. The trial begun on August 2007. So far, a total of 1831 and 1474 patients have been recruited in the microscopy and clinical settings respectively. A total of 3468 patients have also been followed up. The trial is on-going; preliminary results will be reported.

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A STANDARDIZED IGG4 ANTIBODY ELISA FOR DETECTING FILARIAL INFECTIONS AND FOR MONITORING EXPOSURE TO LYMPHATIC FILARIASIS

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The Global Programme to Eliminate Lymphatic Filariasis aims to eliminate LF as a public heath problem in some 80 endemic countries by the year 2020. This ambitious programme is largely based on a strategy of repeated rounds of mass drug administration (MDA). Improved tools are needed for determining endpoints for LF elimination programs and for post-MDA surveillance. Prior studies have shown that an ELISA for IgG4 antibodies to recombinant antigen Bm14 is sensitive for infection or heavy exposure to filarial parasites and that antibody rates in children decrease dramatically after LF transmission has been interrupted by MDA. Thus, antibody surveys of sentinel populations such as primary school children may be useful for confirming interruption of LF transmission after 5 or more rounds of MDA. We now report development of a commercial ELISA test kit manufactured under GMP conditions that employs purified Bm14 antigen with positive and negative calibrator samples for reliable detection of anti-filarial antibodies. The test is more sensitive than a widely used research laboratory version of the test based on this antigen. It reliably detected antibodies in sera from bancroftian and brugian filariasis patients from many different endemic areas. Parallel testing of serum and eluates of dried blood produced equivalent results. Positive tests were not observed with non-endemic normal sera or with sera from patients with no history of exposure to LF who were infected with non-filarial parasites (*Strongyloides*, *Schistosoma*, *T. cruzi*, malaria, dengue and or *Toxoplasma*). However, the test often detected antibodies in sera from patients with onchocerciasis and loiasis. We believe that this test has great potential as a tool for different stages of filariasis elimination programs. For instance, it could be used to map the distribution of filarial infections in endemic countries. More importantly, it is a promising tool for documenting interruption of transmission of new infections following MDA.

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QUALITY OF MEDICAL RECORDS: RESULTS FROM AN AUDIT OF SIX HOSPITALS IN UGANDA

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The goal of the US President's Malaria Initiative is to reduce deaths due to malaria by 50% in 15 African countries, including Uganda. However, measuring malaria mortality in Africa is challenging because medical records are often incomplete, and many deaths occur at home and are not recorded. As part of a study to validate the accuracy of verbal autopsy procedures in determining cause of death in children < 5 years in Uganda, we conducted an audit to assess the quality of medical records in six hospitals from different epidemiological settings (Kampala, Mubende, Tororo, and Kisoro). At each hospital, 100 medical records were randomly sampled from pediatric wards, and were audited by a member of the hospital staff. Data were captured on admission diagnoses, symptoms and signs recorded, diagnostic investigations ordered and recorded, and treatment provided. Of 600 cases evaluated, malaria was the most common reason for admission, with 395 (66%) cases carrying a diagnosis of malaria, severe malaria, or cerebral malaria; respiratory tract infections

(26%) and diarrheal illnesses (13%) were also common. History of the presence or absence of fever associated with the current illness was recorded in most cases (80%), but recording of temperature varied widely; temperature was recorded in most cases at five sites (63 -100%), but at one site, no temperatures had been recorded (p<0.001). Vital signs, including respiratory rate and pulse, were recorded in approximately 50% of cases at one site, but were rarely recorded in the others. Of cases diagnosed with malaria, a blood smear was ordered in 68%, but this varied from 14 - 99% between the sites (p<0.001). Results of the blood smear were recorded in only 65% of cases. Of 154 cases diagnosed with respiratory tract infections, only 65% had a chest exam documented, and again this varied widely between the sites (20 -100%, p<0.001). Good medical records are an essential component of quality health care. Our results suggest that the quality of medical record keeping varies widely between hospitals, and that basic information is often not recorded, decreasing the reliability of medical records in these sites. Interventions to educate health care staff on the importance of medical record keeping and to address identifiable gaps could improve the quality of medical records, possibly improve medical care in Ugandan hospitals, and improve the ability to measure malaria-specific mortality in inpatient settings.

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PHARMACOKINETICS OF SULFADOXINE AND PYRIMETHAMINE IN THE INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY

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Despite extensive use of sulfadoxine-pyrimethamine for intermittent preventive treatment of falciparum malaria in pregnancy (IPTp), knowledge of the disposition of these drugs in pregnancy is extremely limited. Concentrations of sulfadoxine and pyrimethamine are predicted to be lower in pregnancy than in the non-pregnant population due to pregnancy-induced physiologic changes. These changes may have an important impact on the clinical effectiveness of IPTp, especially in the setting of increasing resistance. A prospective, multi-center, self-matched, pharmacokinetic study was conducted in Mali and in Zambia, where IPTp with sulfadoxine-pyrimethamine is part of national malaria control policy. This study was designed to assess pharmacokinetic differences in both sulfadoxine and pyrimethamine between two time periods, during pregnancy and postpartum, with study subjects acting as their own controls. The primary endpoints are total drug exposure (measured as the area-under-the-time-concentration curve). Pharmacokinetic data derived from a total of 43 women from whom filter paper samples were collected at 9 time points over 42 days will be presented. Such data is needed to inform rational dosing of sulfadoxine-pyrimethamine for IPT in pregnancy to optimize its clinical efficacy and safety and potentially extend the useful lifespan of this intervention.

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Q FEVER COMPLICATED BY OGILVIE'S SYNDROME IN A U.S. SOLDIER

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Q fever is a zoonosis caused by *Coxiella burnetii* that occurs worldwide. Although rare in the US, there have been a number of cases diagnosed recently among military personnel deployed to the Middle East. The disease has many clinical manifestations including gastrointestinal symptoms (hepatitis, acalculous cholecystitis, pancreatitis, and mesenteric paniculitis) which can delay the diagnosis. Patients often have multiple laboratory abnormalities during acute disease. We report a case of acute Q fever complicated by Ogilvie's syndrome and review the difficulties in making the diagnosis when multiple laboratory tests are positive. A 29 year old active duty male deployed to Iraq presented with cough and headache. His symptoms increased over the next week and he developed abdominal pain and dark urine. He failed to respond to azithromycin and required hospitalization in Kuwait. He was noted to be anemic and thrombocytopenic requiring red blood cell and platelet transfusions. He had elevated transaminases and hyperbilirubinemia with an unremarkable right upper quadrant ultrasound. He was started on cefotaxime, metronidazole, and ciprofloxacin for cholangitis prior to being transferred to Germany. Q fever was suspected and he was started on doxycycline prior to transfer to Walter Reed Army Medical Center. The patient continued to be febrile and had increased abdominal pain. An abdominal CT scan showed ascites and colonic distention (cecum 9.6 centimeters) without true obstruction consistent with Olgilvie's syndrome. He was treated with neostigmine and his abdominal symptoms resolved. His laboratory results were remarkable for multiple positive tests including: HIV ELISA with indeterminate Western blot, C-ANCA, P-ANCA, Parvovirus IgG and IgM, EBV IgG, CMV IgG and IgM, lupus anticoagulant, and rapid plasma reagin. The patient's Q fever serologies returned positive and he continued the doxycycline. His fevers resolved after 3 weeks and 2 months later the patient is doing well. In conclusion, Ogilvie's syndrome should be added to the list of atypical presentations of Q fever. Immune stimulation during acute Q fever infection can lead to false positive tests for multiple infectious and rheumatologic etiologies. Providers need to be aware of these varying presentations and laboratory findings when considering the diagnosis of Q fever.

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INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN INFANTS IN AN AREA OF HIGH SP RESISTANCE IN TANZANIA: A RANDOMIZED DOUBLE BLIND PLACEBO CONTROLLED TRIAL OF SP, LAPDAP OR MEFLOQUINE FOR PREVENTION OF MALARIA IN INFANTS

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Intermittent Preventive Treatment of malaria in Infants (IPTi) is a promising strategy to reduce the burden of clinical malaria in infants in endemic countries. Six studies using sulfadoxine- pyrimethamine (SP) given to asymptomatic children at time of immunization and other scheduled EPI clinic visits have been published from across different transmission settings and countries in sub Saharan Africa. Efficacy has ranged from non-significant to 60%. Questions remain whether SP IPTi is applicable, efficacious and safe in all transmission settings and particularly in areas of high resistance to SP, and what drug should replace SP should it fail to work. We report the results of a randomized double blind placebo controlled trial of IPTi using SP, chlorproguanil/ dapsone and mefloquine in an area of high resistance to SP in north-eastern Tanzania in 2 different transmission settings (moderate and low).

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MANAGEMENT OF CHILDHOOD DIARRHEAL DISEASE IN GONDAR, ETHIOPIA

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The aim of the study was to understand the management of diarrheal disease by mothers in Ethiopia. Diarrheal disease remains one of the leading causes of death for children under five years of age. More than two million children will die every year from dehydration associated with diarrhea globally. The mothers of 221 children less than five years old with diarrhea visiting health facility in Gondar, Ethiopia were interviewed by community health workers using a structured survey to determine their knowledge and use of oral rehydration therapy. The majority of the mothers reported that they knew how to prepare oral rehydration solution (ORS), yet less than 2% of mothers reported giving ORS or cereal-based solutions to replenish the loss of liquids when their children first displayed signs of diarrhea. Additionally, less than half of the mothers withheld water, tea, rice-water, and juice during episodes, and one out of every five mothers withheld breast milk from their children during diarrheal episodes. There is a disparity between the knowledge and the management of diarrhea in the mothers enrolled in the study. Ethiopian mothers in the study have the knowledge to prepare ORS, but they are withholding fluids and breast milk during the illness. Mothers may have the misconception that providing fluids to their children can exacerbate diarrhea and that giving less fluid may stop the diarrhea. In order to bridge the gap between knowledge and practice, mothers should learn to recognize the early signs and symptoms of diarrhea in their children and immediately increase fluid intake. Community health workers in Gondar should be employed with the skills and knowledge to educate mothers to adequately manage diarrhea, which will be a critical means to mitigate the potential harmful effects of diarrhea and prevent dehydration. A health education intervention was developed and implemented to combat the withholding of fluid from children with diarrhea, a dangerous and potentially fatal practice. A drama group was trained and a pictograph was developed to teach mothers about the importance of increasing fluid intake during diarrhea episodes. Many mothers misinterpreted the messages promoted in the intervention, suggesting research about how mothers perceive health educational materials should be integrated into the implementation of health education programs.

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DIARRHEA OUTBREAK WITH HIGH MORTALITY AMONG YOUNG CHILDREN IN SANTA ROSA, GUATEMALA– 2008

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Diarrhea outbreaks can result in high mortality. From December 2007 through January 2008, during the Guatemala rotavirus season, the Department of Santa Rosa reported 23 diarrhea deaths among children <5 years old, representing a 5-fold increase compared to previous years. We conducted an investigation to characterize these deaths and identify risk factors for mortality. We interviewed parents of the 23 dead children using a standardized verbal autopsy. Children were confirmed as a diarrhea death if they had ≥3 loose stools in a 24-hour period in the 7 days prior to dying. Stool and serum samples were collected when possible and tested for rotavirus and enteric pathogens. We conducted a case-control investigation to identify death risk factors. Controls were diarrhea patients <5 years old admitted to the departmental hospital between December 2007 and February 2008 and prospectively enrolled in an ongoing diarrhea surveillance study. Controls were separated in two groups: rotavirus-positive and rotavirus-negative. Seventeen diarrhea cases were confirmed. Ten (59%) were male. Median age was 4 months (range: 1 month - 4 years). Twenty-nine rotavirus-positive and 15 rotavirus-negative controls were enrolled. Cases were more likely to have signs of dehydration (odds ratio [OR] 3.5; 95% confidence interval [CI] 1.3 - 8.7) and bloody diarrhea (OR 4.5; 95% CI 2.8 - 7.4) than rotavirus-positive controls, and more likely to have signs of dehydration (OR 3.8; 95% CI 1.5 - 10.0) but not bloody diarrhea (OR 2.2, 95% CI 0.8 - 6.1) than rotavirus-negative controls. There was no significant difference in use of oral rehydration solution (ORS) between cases and controls. When compared with rotavirus-positive and -negative controls, deceased children were less likely to have ever been breastfed (OR 0.2; 95% CI 0.1 - 0.4). One whole stool and three serum samples from 4 cases were negative for rotavirus. Four rectal swabs were tested; one (25%) was positive for *Salmonella enterica* type Newport and two (50%) for enterotoxigenic *Escherichia coli*. In conclusion, bacterial pathogens may be associated with diarrheal deaths in rotavirus seasons, but difficulties in obtaining specimens limits identification of all causal pathogens including rotavirus. Introduction of a rotavirus vaccine could help clarify etiologies of increases in diarrhea mortality. Breastfeeding and ORS use should be encouraged to reduce diarrhea mortality.

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BARRIERS TO MAINTAINING THE MICROBIOLOGIC QUALITY OF DRINKING WATER, SOUTH SULAWESI, INDONESIA: BANTAENG AND MAROS DISTRICTS, 2007

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In Indonesia, diarrhea remains a major cause of morbidity and mortality among children <5 years old despite 50 years of intensive government promotion of boiling drinking water. For many populations, boiling is expensive, time-consuming, and environmentally destructive. In preparation for implementation of a point-of-use water chlorination program, we assessed impact of boiling on water quality in South Sulawesi. We surveyed a random sample of households with at least one child <5 years old in 39 villages in 2 rural districts about demographic and socioeconomic characteristics, water sources, storage, and treatment practices. From a random sample of 20% of households, we tested source and stored water samples for *Escherichia coli* contamination. We enrolled 1,205 households with 1,468 children <5 years old. Seventy-one percent of respondents used tap water; all stored water in the home and 84% reported boiling drinking water. Water samples were obtained from 242 households; 96% of source and 51% of stored water samples yielded *E. coli* (44% of boiled water samples versus 89% of unboiled samples; 44% of richest two socioeconomic quintiles versus 56% of poorest three quintiles). Water that was reportedly not boiled (RR=2.0, 95% CI=1.7-2.5), was stored in wide-mouthed (RR=1.4, 95% CI=1.1-1.8) or uncovered (RR=1.8, 95% CI=1.3-2.4) containers, or was stored in households in the three poorest quintiles (RR=1.4, 95% CI=1.1-1.9) was more likely to yield *E. coli*. A multivariate model, controlling for district, showed that water contamination was independently associated with not boiling (OR=6.8, 95% CI=3.2-14.4) and being in the three poorest quintiles (OR=1.8, 95% CI=1.1-3.1). In conclusion, although *E. coli* contamination of stored water was more frequent in households that did not boil and were poor, contamination was also prevalent in households that did boil and were in the richest two quintiles. Chlorine-based water treatment, which is less expensive, and safer water storage containers offer an alternative approach to disease prevention.

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AVAILABILITY OF ORAL REHYDRATION SOLUTION PACKETS AT SMALL SHOPS IN TWO AREAS OF KENYA, 2007

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Recent data indicate that treatment of childhood diarrhea with oral rehydration solution (ORS) is declining in Kenya. Small shops that sell routine household goods can be a community resource for health products such as ORS. We sought to identify factors associated with ORS availability at small shops to inform efforts to improve community-level diarrhea case management. The study included all small shops in two study sites: 33 villages of Asembo, a rural area in western Kenya, and all small shops in the 2 villages of Kibera, a Nairobi slum. Using a structured questionnaire, we conducted a cross-sectional survey of one worker per shop. We performed logistic regression to identify factors associated with availability of ORS at the shop on the interview day. We interviewed 134 shop workers in Asembo and 233 in Kibera. Overall, 40% of shop workers identified themselves as pharmacists. However, only 3% of those identifying themselves as pharmacists said they had formal pharmacy training. Of shops visited, 10% had ORS packets available for sale on the interview day, including 4% of Asembo shops and 13% of Kibera shops (p=0.004). Antimicrobial drugs were reported to be usually available at 33% of shops. In multivariate analysis, self-identification as a pharmacist (ORadj= 20.1, 95% CI= 7.2-56.6), having antimicrobial drugs usually available (ORadj= 6.8, 95% CI= 2.6-17.6), and telling customers that ORS is a medication (ORadj= 5.1, 95% CI= 1.5-17.2) were significantly associated with ORS availability on the interview day. In conclusion, to improve diarrhea case management at the community level, ORS should be made widely available through small shops in Kenya. Shop workers should be trained to recognize the effectiveness of ORS for diarrhea treatment and the importance of widespread distribution of ORS through small shops. Minimizing the availability of antimicrobial drugs at small shops in Kenya may reduce inappropriate antimicrobial use.

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RECOMMENDATION OF ORAL REHYDRATION SOLUTION FOR DIARRHEA CASE MANAGEMENT BY PHARMACY WORKERS IN KENYA, 2007

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In Kenya, use of oral rehydration therapy, including oral rehydration solution (ORS), declined by 32% from 1998 to 2003. Pharmacies are important sources of information and therapeutics for parents of children with diarrhea. We assessed factors associated with ORS recommendation by pharmacy workers in rural Asembo, in western Kenya, and urban Kibera, a large Nairobi slum. We enumerated all pharmacies in the study sites and interviewed one worker at each pharmacy. We inquired about pharmacy workers' treatment recommendations for their most recent case of pediatric watery diarrhea with some or no dehydration, as defined by WHO. We performed logistic regression to identify factors associated with ORS recommendation. We interviewed 155 workers, 23 (15%) from Asembo and 132 (85%) from Kibera (median age = 29 years [range 17-56]). Of 88% who reported being trained pharmacists, the median number of years of training was 3 (range 1-5). The most effective treatment for watery diarrhea was reported to be ORS by 42%, and antimicrobial drugs by 34%. On the interview day, ORS was available in 72% of pharmacies surveyed in Kibera and 48% of pharmacies surveyed in Asembo. Of 83 workers who reported being consulted recently for a case of pediatric watery diarrhea, 69% reported recommending ORS and 81% reported recommending antimicrobial drugs. Belief that death can be a complication of watery diarrhea (OR_{adj}=7.9, 95% CI=1.6 - 39.7) and the number of years worked in the pharmacy (OR_{adj} =1.6, 95% CI=1.1-2.2) were independently associated with ORS recommendation. In conclusion, ORS was unavailable at nearly 1/3 of Kenyan pharmacies surveyed; availability was lower in the rural area. Interventions to improve diarrhea case management must ensure universal availability of ORS at pharmacies and emphasize the risk of death from dehydrating diarrhea to pharmacy workers. All pharmacy workers, including those without formal training, should be targeted for education to increase ORS recommendation and to decrease inappropriate use of antimicrobial agents for diarrhea treatment.

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INAPPROPRIATE RECOMMENDATION OF ANTIMICROBIAL AGENTS FOR TREATMENT OF WATERY DIARRHEA BY HEALTH WORKERS IN KENYA, 2007

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Use of antimicrobial agents creates a selective pressure for dissemination of antimicrobial resistance. Understanding the current patterns of antimicrobial use for treatment of non-severe watery diarrhea is needed in Kenya. We assessed knowledge, attitudes, and treatment practices for non-severe watery diarrhea in a cross-sectional survey of Kenyan health workers (HWs) aged ≥18 years in Kibera, a Nairobi slum, and in rural Asembo. We asked HWs to recommend treatment for a hypothetical pediatric case of non-severe watery diarrhea with some dehydration, according to the WHO definition of diarrhea. We interviewed 333 HWs, including 28 (8%) clinicians (medical officers, nurses, clinical officers), 118 (37%) community health workers (CHWs), and 125 (38%) herbalists, traditional healers, spiritual healers and village doctors. Many (49%) respondents believed that antimicrobial agents kill viruses (49%), prevent dehydration (47%), and treat dehydration (35%). For a hypothetical patient with non-severe watery diarrhea, 12% recommended antimicrobial therapy, including 57% clinicians, 11% of CHWs and 1% of herbalists and traditional healers recommended antimicrobial agents. After adjusting for HW type, HWs who believed that antimicrobial agents treat dehydration were more likely to recommend them (OR=2.8, 95% CI 1.1-7.2). HWs who believed antimicrobial agents were very expensive were less likely to recommend them (OR=0.4, 95% CI 0.1-1.0). Of the 40 HWs who recommended antimicrobial agents, 50% stated the diarrhea in the hypothetical case was likely caused by bacteria and 93% also recommended oral rehydration therapy (ORT). In conclusion, HWs in Kenya, particularly clinicians, overestimate the effectiveness of antimicrobial agents. Knowledge of ORT appears to be high among those who recommended antimicrobial agents. Increased education about limitations and possible negative consequences of antimicrobial therapy for non-severe diarrhea is needed in this setting.

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FECAL LEUCOCYTES IN DIARRHEAGENIC *E. COLI*

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Diarrheagenic *E. coli* are the most commonly isolated enteric pathogens in children in developing countries. However, there are few data on the level of fecal leucocytes as a marker of inflammatory response in children infected with these pathogens. The purpose of this study was to determine the presence and quantity of fecal leucocytes in diarrheagenic *E. coli* cases and to compare these levels between diarrhea and control samples without diarrhea. Stool samples from diarrhea episodes and from randomly selected healthy controls were analyzed from a cohort study of 1025 children younger than 1 year of age in Lima, Peru. Stools were analyzed for common enteric pathogens and five *E. coli* colonies/patient were studied by a multiplex real-time PCR to identify: Enterotoxigenic (ETEC), Enteropathogenic (EPEC), Shiga toxin-producing (STEC), Enteroinvasive (EIEC), Enteroaggregative (EAEC), and Diffusely Adherent *E. coli* (DAEC). Stool smears were stained with methylene blue and read by a blinded observer. The number of polymorphonuclear leucocytes per high power field (1000X) (L/hpf) was determined in at least fifty fields; the results were categorized as ≤10 L/hpf or >10 L/hpf. We have analyzed 1164 smears from 810 diarrhea cases and 354 controls. Fecal leucocytes >10L/hpf were present in 13% (102/810) of all diarrheal episodes vs. 1% (4/354) of controls (p<0.001). Diarrheagenic *E. coli* were isolated in 23% (189/810) of diarrhea samples and 28% (99/354) of controls. Among stool samples with diarrheagenic *E. coli* as the only pathogen isolated (excluding co-infection with other bacteria or viruses) fecal leucocytes >10L/hpf were present in 10% (18/189) (11-20L/hfp [7%], 21-49L/hpf [2%], ≥50L/hpf [1%]) of diarrhea vs. 1% (1/99) of controls (p<0.01). Among diarrheal samples, fecal leucocytes >10L/hpf were present in 25% (1/4) of STEC cases, 19% (3/16) of ETEC, 17% (3/18) of DAEC, 9% (4/46) of EPEC and 8% (7/93) of EAEC. In conclusion, although diarrheagenic *E. coli* were isolated with similar frequency in diarrhea and control samples, clearly they were associated with more inflammatory response during symptomatic infection in small children. In general, these pathogens elicited a mild inflammatory response.

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A NOVEL NEONATAL MURINE MODEL OF ENTEROAGGREGATIVE *ESCHERICHIA COLI* INFECTION

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Enteroaggregative *Escherichia coli* (EAEC) is an emerging enteric pathogen that causes acute and chronic diarrhea and malnutrition among children, adults and those that are immunocompromised in developing and even a leading bacterial pathogen that causes diarrhea in developed countries. We have evaluated the use of neonatal C57BL/6 mice as a model of EAEC infection. Cohorts of 6 day-old mice (N = 12) were infected via oral inoculation with 10^6 cfu/mouse of the pathogenic EAEC 042 wild type (WT) strain; a mutant EAEC 042AggR; the non-pathogenic HS strain; or culture medium (DMEM). One cohort was undisturbed and was observed for growth and bacterial shedding in stool. The remaining cohort was euthanized on Days 6, 9, 12 and 15 post-infection for histology and assessment of intestinal colonization. Detection of the test organisms was done by real-time PCR probing for the plasmid-borne *aap* gene. Nourished mice challenged with 042 WT showed impaired growth compared to the DMEM group from Day 1 up to Day 27 post infection (p<0.001-0.008), to the HS-challenged group (p<0.001-0.021), as well as to the 042AggR group (p<0.001-0.019). Stool shedding in 042-challenged mice using real-time PCR was chronic, up to 3 weeks after infection, and colonic tissue was qPCR-positive for 042 organisms as well. HandE staining of ileum and colon in 042-challenged mice showed mild inflammation, disrupted epithelium, and the presence of bacteria inside mucus-containing vacuolated goblet cells in the colon. Further studies showed a) ability to infect malnourished neonatal but not nourished adult mice; b) a relationship of challenge dose and growth shortfalls; and c) similar results with EAEC strain JM221, resulting in growth shortfalls (p<0.001-0.043) compared to DMEM treated group in pre-and post-weaning. Enteroaggregative *Escherichia coli* has emerged as an important pathogen causing diarrheal disease in multiple epidemiologic and clinical settings. Our neonatal C57BL/6 murine model opens opportunities for studying pathogenesis and testing potential therapeutic agents as well as host and pathogen traits to better understand their roles in causing disease.

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ROTAVIRUS INFECTION PREVALENCE AMONG INDONESIAN PEDIATRIC GASTROENTERITIS PATIENTS

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During the last decade, with the development of newer technology, viruses were being identified as another important cause of diarrhea among pediatric patients, even in the developing countries. This study was undertaken to estimate the Rotavirus enteritis prevalence among pediatric diarrhea patients in Indonesia. Passive surveillance of diarrhea among pediatric patients visiting hospitals and health centers in six cities of Indonesia in 2006. Stool samples/rectal swabs were placed into Cary-Blair transport media for bacterial isolation and in a sterile container for viral identification. A total 9,954 patients comprised of 5,881 boys and 4,073 girls (ratio 1.4:1), with ages 1 month - 6 years, participated in the study and provided stool/rectal swab samples. Total of 4,617 samples were examined for Rotavirus, and showed that 2,394 (52%) were positive for Rotavirus Croup A. A total of 778 (7.8%) samples were positive for bacterial isolates, however 150 (69.8%) of 215 samples that were positive for bacterial isolates were also positive for rotavirus examination. In conclusion, fifty two percent (52%) of the samples examined were positive for Rotavirus. This information is important for pediatric health care providers treating children with non-dysenteric diarrhea.

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THE IMPACT OF A COMMUNITY-BASED PROGRAM TO PROMOTE POINT-OF USE WATER CHLORINATION PRODUCTS AND REDUCE DIARRHEAL RATES IN RURAL WESTERN KENYA

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Approximately 1.7 million children < 5 years old (yo) die annually due to diarrhea. Consuming unsafe drinking water contributes substantially to these deaths. Point of use (POU) chlorination products for household drinking, which are socially marketed in Kenya, have been shown to reduce diarrhea risk; however, use rates remain low. The Nyando Integrated Child Health and Education (NICHE) project promotes POU chlorination through women's groups, schools, clinics, and churches in rural villages in western Kenya to motivate water treatment behaviors and decrease diarrhea rates in children < 5 yo. We evaluated the impact of

the NICHE project on POU chlorination and diarrhea rates by randomly selecting 60 villages and allocating them into intervention and comparison groups. The intervention villages received the NICHE and social marketing activities while the comparison villages received only social marketing. We visited a random sample of households biweekly to collect data on use of water treatment products, chlorine residuals in stored water, and diarrhea episodes. We collected data from 1395 households with 1750 children < 5yo. Respondents' median age was 30 years (range 16-90y); 84% had a primary school education or less and 86% were in the poorest socioeconomic quintile. Over 16 rounds of biweekly home visits, a median proportion of 20% (range 11-34%) of intervention households and 14% (range 7-43%) of comparison households reported chlorinating stored water the day of the visit. Measurement of residual chlorine in stored water confirmed chlorination in 13% of all intervention home visits and 12% of comparison home visits (p>0.05). The median proportion of children < 5yo with diarrhea in the preceding 24h over 16 rounds was 4% in both intervention and comparison groups. Although reported POU water treatment was higher in intervention than comparison households, confirmed chlorination and diarhea rates were similar in the two groups, suggesting that, in the first 8 months of the project, the impact of NICHE interventions did not exceed that of social marketing alone. Biweekly home visits will continue for another year to assess the impact of the NICHE project on behavior change and diarrheal rates over time.

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ARGININE REDUCES *CRYPTOSPORIDIUM PARVUM* INFECTION IN MALNOURISHED SUCKLING MICE WITH INDUCIBLE NITRIC OXIDE AND ARGINASE INVOLVEMENT

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Cryptosporidium parvum is an enteric-based protozoan spread by fecal-to-oral contact that causes debilitating diarrhea in its host. Arginine deficiency has been associated with severe catabolic states. L-arginine is a substrate to nitric oxide synthase (NOS) and arginase isoforms. Malnutrition was achieved by separating the pups from their mothers (4 h at D4, 8 h at D5 and 12 h from D6 to D13). The following treatment regimens were initiated on the 4th day of life and given subcutaneously daily: either 100mM or 200mM of L-arginine; and PBS for the *C. parvum*-infected controls. Groups treated with 200mM L-arginine were subdivided receiving either 20mM of NG-nitroarginine-methyl-ester (L-NAME) or 2mM of S-(2-boronoethyl)-l-cysteine, BEC. For Crypto infection, each pup received an oral inoculum of 10^6 excysted *C. parvum* oocysts in 10µl of PBS on day 6. Treatment with 200mM of L-arginine significantly improved weight gain compared to the untreated infected control. L-NAME treatment (20mM) profoundly impaired the relative body weight gain over the same period, reducing weight gains as compared to the infected PBS control. Cryptosporidial infections were associated with crypt hyperplasia, villus blunting and inflammation. Arginine treatment resulted in near normalization of mucosal histology. BEC completely abrogated the arginine-induced improvements, while L-NAME had less of an effect on the histological improvements as seen with L-arginine alone. We found a high expression of iNOS in the ileum of malnourished uninfected mice. Infected PBS control mice showed an intense expression of iNOS as well. Both L-arginine treatments reduced oocyst shedding per mg of ileal tissue (p<0.001). L-NAME (20mM) and BEC (2 mM) significantly reversed the effects of L-arginine (p<0.05). *C. parvum* infection significantly increased urine NO_3^- and NO_2^- concentration when compared to non-infected controls. L-arginine treatments significantly increased nitric oxide metabolites when compared to infected and non-infected controls. The combined treatment of 200mM of L-arginine and L-NAME reversed this observed effect. The combination of 200mM of L-arginine and BEC dramatically increased the concentration of NO_3^- and NO_2^- in the urine when compared to all treatment regimens. Altogether these findings suggest a protective role of L-arginine during *C. parvum* infection in malnourished growing mice.

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TYPHOID FEVER: A RARE CAUSE OF FEVER AND DIARRHEA IN USA

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Typhoid fever affects roughly 21 million people annually causing nearly 600,000 deaths. The causative organism is a gram negative enteric bacillus *Salmonella typhi* of family Enterobacteriacae. The incidence of typhoid fever in USA is low (<400 cases per year) mostly (75%) among travelers but poses a risk of emergence. 25 years old Hispanic male with no past history presented with complaints of fever, abdominal pain and diarrhea. 2 weeks ago he started having moderate to high-grade continuous fever associated with chills, rigor and headache. After one week, the patient started having non-bloody diarrhea, 6-7 stools per day associated with generalized abdominal pain. Review of systems was negative. No travel or recent antibiotic use. Patient recalled eating cheese sandwich and sausages 3 days prior to the fever from a local deli. On examination the patient had diffuse abdominal tenderness with no guarding or rigidity. Antibiotics were started for suspected bacterial gastroenteritis. Blood cultures were positive for gram-negative rods, later confirmed to be *Salmonella typhi*. Stool studies were negative. Initial antibiotics were changed based on culture susceptibility. The patient improved after 8 days, and was discharged on oral antibiotics. Contaminated drinking water and food are the major source of transmission in developing countries. The etiology of most of the cases in United States not related to travel abroad could not be accounted for. Chronic carriers of *Salmonella typhi*, especially among immigrant population, are a major risk factor for the spread of the disease. Without antibiotics the mortality rate for typhoid fever is about 20% and with prompt therapy mortality is reduced to less than 1%. 2-5% of infected patients become carriers**.** Identification of chronic carriers and their treatment either with antibiotics or surgically is essential in industrialized nations with low incidence of typhoid fever. Chronic carriers should avoid handling foods. Sanitation and hygiene are critical measures that can be taken to prevent typhoid fever. Patient must be reported to the local health department and food handler should be prohibited from work until proven free of the organism.Travelers should avoid raw leafy vegetables and foods stored at room temperature.Vaccination is recommended at least one week prior to travel to endemic area.

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ETIOLOGY OF TRAVELERS' DIARRHEA AMONG INTERNATIONAL TOURISTS IN CUSCO, PERU

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Peru is an important travel destination worldwide and every year receives >1 million tourists, most traveling to Cusco and Machu Picchu. We studied the etiology and impact of Traveler's diarrhea (TD) among tourists seeking medical attention in Cusco. We enrolled adult travelers presenting to three private physicians with 3+ unformed stools during <72 hours. Subjects who received antibiotics or had a history of chronic diarrhea were excluded. Participants completed an epidemiologic questionnaire and provided a stool sample. Bacterial enteropathogens were identified by culture and antibiotic sensitivity tests were performed by disk diffusion

method. Parasites were identified by ELISA. We estimated the frequency of each pathogen and described the impact of TD. We enrolled 206 travelers, mainly from Holland, (23%), the U. K. (20%) and the U. S. (15%), from June 2003 to May 2008; ave. age 26yrs (range 18-76yrs) and 66% female. Of all subjects, 41% reported fever, 32% had vomiting, and 10% had bloody stools. *Campylobacter* spp (*C*spp) and *Shigella* spp (*S*spp) were found in 25 (12%) and 14 (7%), respectively. Enterotoxigenic *Escherichia coli* (ETEC) was isolated in 24 subjects (12%), with most ST+ only (15, 62%) or LT+ only (5, 21%). *Cryptosporidium* spp was found in 20% (41) and *Giardia lamblia* in 4.4% (9). Bacteria/parasite co-infection with found in 18% and 65% had at least one pathogen, Ciprofloxacin resistance among *C*spp was 74% (14/19, 95% CI: 49%-91%) but only 0-7% for other bacteria. Trimethoprim-sulfamethozaxole resistance was 35% for *ETEC* and >90% for *C*spp and *S*spp. Most travelers (85%) reported that TD affected their activities, mainly by staying in bed (61%), missing tours (24%) or changing itineraries (12%). Our results highlight the burden of TD on international tourists, both due to the symptoms and the disruption of travel plans. High levels of *Cspp* resistance to fluoroquinolones indicates a need for further surveillance and potentially adjustment of prescription policies. Prevention strategies including vaccines targeting *Campylobacter*, ETEC and *Shigella* infections may be appropriate for international travelers.

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PROLONGED DENGUE OUTBREAK IN TOURIST AREA, AN EASTERN PROVINCE, THAILAND, MARCH - JULY 2007

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Dengue has been an endemic disease in Thailand for decades and it mainly affected at hyper-endemic area with multiple dengue serotypes circulation. On June, 2007 Rayong Health Office notified Bureau of Epidemiology due to prolonged dengue outbreak for more than 70 days. We conducted outbreak investigation aimed to initially identify the magnitude of the dengue outbreak, second to identify risk factors of dengue infection and finally to evaluate and implement control measure. Active case finding was conducted in the highest affected villages (2and3) at Phae Sub-district by cross-sectional dengue seroprevalence survey in both villages was conducted. A case definition was applied from the WHO. Environmental and entomological investigations were carried out. Cross-sectional analytic study was done for identify risk factors by univariate and multivariate analysis. Phae Sub-district was highest morbidity rate (360.31/100,000 pop.).There is a tourist area and Cambodian immigrants moving in and out. The mosquito larvae Breteau index was high in village 2 and 3 (210 vs. 245). Overall 223 blood samples were testing for dengue by ELISA and 16 cases (7.2%) revealed positive. Median age was 15 years (1-39). High attack rate in Cambodian immigrant (81.2%) was observed. Dengue fever (81.2%) and asymptomatic (18.8%) were presented. Acute and recent infections were 68.8% and 31.2% respectively. Secondary infection was 50%. Dengue infection rates were 81.2% in Village 2. The risk factors for acquiring dengue infection included visiting dengue case, having a household member with dengue infection and Cambodian workers. Multiple logistic regression showed visiting dengue case (adj. OR 14.9; 95% CI 3.4-64.8) and Cambodian workers (adj.OR 7.2; 95%CI 1.4-36.6). There was all 4 dengue serotypes circulation from Rayong Hospital data in 2007. This dengue outbreak investigation revealed hyper-endemic area and multiple dengue serotypes. Prolonged outbreak was due to high people migration, high mosquito indices and occult infection in immigrant worker who not reached to reporting system. Thus Cambodian were the independent risk of dengue infection due to their over crowding habitat. And Dengue case visiting was strongest risk for dengue infection because of hyper-density of *Aedes* mosquito surrounding patient's habitat. Intensive investigation and control during first few dengue cases were crucial role for preventing the prolongation of outbreak.

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PRODUCTION AND CHARACTERIZATION OF SINGLE-CYCLE REPLIVAX VACCINES TO PREVENT DENGUE

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Efficacious vaccines are needed for several flavivirus diseases, including dengue. We have previously described a novel flavivirus vaccine technology based on genetically engineered single-cycle flaviviruses. Our vaccine, named RepliVAX, shares some properties with live-attenuated vaccines (LAVs), since it can infect cells in vaccinated animals. However these infections cannot spread or cause disease due to a deletion in the C gene, making RepliVAX safer than other LAVs. Despite its inability to spread among normal cells, RepliVAX can be grown to high titer in cell lines that express high levels of C, in a production format that is similar to that used to produce LAVs. When RepliVAX infects normal cells it produces high levels of protective immunogens. We have recently shown that RepliVAX WN (derived from WNV) can be used as a "vaccine platform" by demonstrating that a "chimera" created by substituting the prM/E genes of RepliVAX WN with the same genes of JEV can produce a JE vaccine, as described previously. Here we report that we have adapted our RepliVAX technology to produce a dengue vaccine by replacing the prM/E genes of RepliVAX WN with the same genes of dengue virus type 2 (DENV2). Our first RepliVAX construct, referred to as RepliVAX D2, replicated poorly in C-expressing cells. However, by adding back a fragment of the deleted C gene, and transfecting this derivative into C-expressing cells and co-cultivating these cells with C-expressing cells for 8 passages we were able to generate a better-growing version of this construct. Sequence analyses of the "adapted" genome revealed amino acid changes in prM and E which were used to produce RepliVAX D2.2, which displayed acceptable growth in C-expressing cells. When RepliVAX D2.2 was used to vaccinate AG129 mice (an immunodeficient murine model for dengue), it produced dose-dependent DENV2-neutralizing antibody responses, and the vaccinated mice were protected from challenge with DENV2. Taken together these studies demonstrate that RepliVAX technology can be applied to dengue vaccines.

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THE INFLUENCE OF CLIMATE ON DENGUE TRANSMISSION AT LOCAL AND REGIONAL SCALES IN PUERTO RICO

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Precipitation stimulates egg hatching and provides oviposition sites for *Aedes aegypti*, the principal vector of dengue viruses. Increased temperature meanwhile increases their development rate, decreases the length of their reproductive cycle, and increases their ability to transmit the virus. Though all of these effects may contribute to increased transmission of dengue, the relationship between climate and observable dengue transmission patterns is less clear. Models for different ecological settings have elucidated widely differing effects. Some of these differences arise from inherent multicollinearity and unaccounted for autocorrelation. Others likely arise due to the diverse ecological settings in which the studies take place even though the underlying transmission system is presumably universal. We adjusted for multicollinearity and autocorrelation using spline smoothing and used a multi-level analysis to analyze the effects of climate effects on local and island-wide scales in Puerto Rico. Temperature and precipitation had overall positive effects on dengue transmission 0-3 months later. In particular localities however these effects

were modified by local climate characteristics. In areas where mean precipitation is particularly high, for instance, the contribution of changing precipitation is minimized. Our results suggest that dengue transmission in Puerto Rico is influenced by climate and that the spatial heterogeneity of observed effects relates to local climate characteristics rather than differences in the fundamental dynamics of transmission.

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ECONOMIC COST OF OFFICIALLY REPORTED DENGUE CASES IN EIGHT COUNTRIES IN THE AMERICAS AND ASIA

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Despite the growing worldwide burden of dengue fever, the global economic impact of dengue illness is poorly documented. Using a common protocol, we present the first aggregate estimates of the economic cost of dengue cases in eight American and Asian countries. We conducted prospective studies of the cost of dengue in five countries in the Americas (Brazil, El Salvador, Guatemala, Panama, and Venezuela) and three in Asia (Cambodia, Malaysia, and Thailand). All studies followed the same core protocol with interviews and medical record reviews. The study populations were patients treated in ambulatory and hospital settings with a clinical diagnosis of dengue. Most studies were performed in 2005. Costs are in 2005 international dollars (I$) and US dollars (US$). We studied 1,695 patients (48% pediatric and 52% adult); none died. The average illness lasted 11.9 days for ambulatory patients and 11.0 days for hospitalized patients. Among hospitalized patients, students lost 5.6 days of school, while those working lost 9.9 work days per average dengue case. Overall mean costs were I$514 and I$1,394 per ambulatory and hospitalized case, respectively. With an annual average of 574,000 cases reported, the aggregate annual economic cost of dengue of officially reported cases for the eight study countries is I$587 million or US$238 million. Preliminary adjustment for under-reporting could raise this total to $1.8 billion, and incorporating costs of dengue surveillance and vector control would raise the amount further. In conclusion, dengue imposes substantial costs on both the health sector and the overall economy.

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SPATIAL VULNERABILITY TO DENGUE IN COASTAL THAILAND: A CASE STUDY, PRACHUAP KHIRIKHAN, 2003-2007

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Dengue is one of the most important vector-borne diseases in Thailand, with increasing annual number of cases despite of control effort. There exists complex temporal and spatial heterogeneity in the vulnerability among different geographic area, with important implication in epidemic control. There have been much progress in the use of geographic information system (GIS) for disease mapping and identifying risk area in recent years. However, previous studies were limited to the district- or municipality-level analysis. The present study aimed to analyze the temporal and spatial clustering pattern of dengue in the sub-district (Tambon) level in coastal Thailand, Prachuap Khirikhan. We obtained the time and location data of reported dengue cases in Prachuap Khirikhan province during 2003-2007. Population statistics in year 2003 were used to calculate incidence rate in each Tambon. Then five years dengue incidences were mapped and local Moran's I index was used to identify spatial cluster. There exists a stable clustering pattern, with significant correlation of dengue incidence among neighboring areas (local Moran's I = 0.0004-0.0006, Z-score > 1.96), with the exception of the two hyperendemic area: Tambon Rontong (annual dengue incidence 1613.38/100,000 in 2007) and Tambon Nongkae (annual incidence 708-1,613/100,000) where the dengue incidence was the highest in the province and did not correlate with that in neighborhood area. In conclusion, our findings show a spatial heterogeneity in the vulnerability to dengue in sub-district level, and demonstrate the potential usefulness of GIS technology in identifying the high risk area of dengue transmission. Comparison of the environmental and social factors between the high risk area and low risk area could shed light on risk factors and potential targets for an effective public health intervention.

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TRENDS OF DENGUE CASES AND DEATHS IN CEBU, PHILIPPINES

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In an attempt to examine the incidence pattern of dengue infection in Cebu, Philippines, we analyzed all the dengue cases recorded at different Regional and Surveillance Unit (RESU) sentinel hospitals of the Department of Health, Central Visayas (Region 7). In the last six years (2002-2007), a total of 17,675 dengue cases and 442 deaths were reported in 51-54 municipalities or cities in Cebu Province. Cebu City had the highest reported dengue cases throughout this period (mean per year = 1,134 cases and 28 deaths; range per year = 605-1,670 cases and 8-57 deaths). Mandaue City ranked second in 2002-2004 and in 2007 (mean per year = 312.5 cases and 6 deaths; range per year = 226-418 cases and 2-14 deaths). Both sexes were almost equally represented. More data will be retrieved, analyzed and presented. Public health officials have recommended the 4 S "*Kontra*" dengue control program to the local government units, local health units, district hospitals, school authorities and the general public. The program includes search and destroy mosquito breeding sites, self-protection measures, seek early treatment, and say "no" to indiscriminate fogging.

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EVALUATION OF NS1 PROTEIN DETECTION AS A DIAGNOSTIC METHOD FOR DENGUE-3 INFECTION IN BRAZIL

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Dengue viruses (DENV) are the most important arboviruses of public health significance. These viruses usually cause dengue fever, but some patients experience a more severe form of the illness, the dengue hemorrhagic fever/dengue shock syndrome. Routine dengue diagnosis can be accomplished through virus isolation, molecular detection of viral RNA, or serologic methods. The diagnosis is usually done by an IgM-capture ELISA (MAC-ELISA) on convalescent samples, but our experience shows that many patients do not return to collect the sample that would ultimately make the diagnosis. For this reason, a diagnostic test capable of making the diagnosis during the acute phase of the disease is of paramount importance to a good disease management that would result in reduction in disease complications and mortality rates. Based on these assumptions, this study evaluated the accuracy of NS1 detection compared to virus RNA

detection by RT-PCR, serology and viral isolation on two-hundred and fifty samples of patients suspected of having dengue. Dengue diagnosis was accomplished in 81 (32.4%), 34 (13.6%), 86 (34.4%), and 22 (8.8%) patients by RT-PCR, viral isolation, NS1 and IgM detection, respectively. Only dengue-3 virus was detected either by RT-PCR or viral isolation. Taking the combination of any of the traditional tests that would make the dengue diagnosis as the gold-standard for the diagnosis, the NS1 test sensitivity was 77.4% and specificity 97.2%, higher than any other method tested. The positive predictive value in this case was 95.4%, and the negative predictive value was 84.6%. NS1 antigen detection presented the best results among the diagnostic techniques applied in this study. The test was also able to detect dengue virus infection longer than RT-PCR and shortly after the beginning of disease symptoms. Based on our results, NS1 antigen detection should be used routinely for dengue diagnosis.

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VIRUS REPLICATION IS INHIBITED BY RNA INTERFERENCE (RNAI) DIRECTED TO DIFFERENT DENGUE GENOMIC REGIONS

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Dengue is considered the most important arboviral disease of humans. It is estimated that 100 million dengue cases occur every year around the world. The need for a safe and efficient approach either for treatment or prevention has been a global priority. However, neither an effective drug nor a vaccine is available for human use. In this study, the influence of short hairpin RNAs (shRNAs) on DENV-2 infection was analyzed in HepG2 and U937 cells. RNA interference (RNAi) is a specific phenomenon of gene silencing carried out by either endogenous or exogenous double strand RNA [(ds) RNA] molecules. The DENV-2 genome target sequences chosen to gene silencing (5' UTR, 3' UTR and prM) were selected by sequence alignment of all four serotypes of the virus, and the homologue sequences were selected to construct (ds) RNAs and cloned into the pSilencer®. Plasmids expressing the RNAi target and RNAi negative control were transiently transfected into U937 cells and constitutively transfected into HepG2 cells, and then infected with DENV-2 at a multiplicity of infection of 0.1 and 0.5. Cell supernatants were collected at 24, 48, and 72 hours after infection. Gene silencing on transiently transfected cells, measured as the decreasing in dengue virus RNA copies, was observed, but only at 48hs post-infection what indicated the loss of cell phenotype induced by the presence of the vector expressing the shRNAs. On the other hand, the constitutively transfected cells induced a long-lasting inhibition of viral replication. Based on these results, we believe that RNAi can be used to inhibit dengue virus replication and the combined use of other areas of the flavivirus genome susceptible to gene silencing could bring this infection under control.

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MOLECULAR EPIDEMIOLOGY OF THE FOUR SEROTYPES OF THE DENGUE VIRUS IN PERU DURING 1,990 - 2,007 AND MOLECULAR SURVILLANCE IN 2,008

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Dengue fever (DF) is the arbovirosis of greater importance in public health. In Peru, the first cases of dengue was reported in the Amazonian in 1,990, since then, dengue has extended from the East to West and of North to South having to the date the circulation of four dengue virus (DENV) serotypes and the presence of cases of Dengue Hemorrhagic fever (DHF). This study was undertaken to identify the circulation of different genotypes of the DENV serotypes in Peru from 1,990 to the present time and its association with DHF and to establish a system of molecular surveillance for serotyping and genotyping, respectively. Ninety dengue virus isolated that correspond to different region of Peru and collected during 1,990 to 2,007 were processed. The sequence analyzed was the E/NS1 portion with primers previously described by Domingo C. The results showed the circulation of genotypes clearly defined: America Africa genotype (DEN1), America and America-Asia genotypes (DEN2), India genotype (DEN3) and Indonesia genotype (DEN4). The DHF in Peru is related to the genotypes America-Asia genotype (DEN2) and India genotype (DEN3). The circulation of serotypes was in different years and the first co-circulation of all serotypes was in 2,001, during this time the first cases of DHF appeared in the North of Peru. In 2,008 the circulation of all serotypes is continuous, in the North coast the circulation of DEN1, 3 and 4 serotypes is reported, DEN2 is circulating in the South east. The molecular analysis shows similarity with genotypes from Colombia, Venezuela and Brazil, respectively. The DHF cases are related India genotype (DEN3) that shows dispersed cluster.

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THE EFFECT OF ROAD ACCESS ON THE TRANSMISSION OF DENGUE FEVER IN RURAL ECUADOR

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The Centers for Disease Control describes dengue as the most important mosquito-borne viral disease affecting humans. Due to the domestic breeding and feeding habits of the *Aedes* vector, dengue is considered an urban disease. The epidemiology of dengue in rural areas, therefore, is not well understood. This study describes dengue seropositivity in rural villages in northern coastal Ecuador, specifically examining the association between road access and the levels of human dengue antibodies. In July and August 2007, bloodspots via finger-prick were collected from 642 of 1,670 individuals (38%) from six villages-- three villages reside along a road and three reside along a river with no road access. The presence of dengue IgG antibodies was tested using ELISA techniques. Among the three road villages, 259 of 334 villagers (78%) tested positive for dengue IgG antibodies, while among the three non-road villages, 118 of 308 (38%) tested positive. Furthermore, seropositivity levels significantly decrease with decreasing access to roads. Analysis of the age stratified serology data provide information on the historical transmission patterns of dengue in this rural setting. These data document high levels of dengue in rural areas. The significant trend with decreasing road access, suggests that movement and migration patterns between these rural communities and neighboring urban centers may explain these data. Further studies are now needed to examine the role of migration on determining our observed transmission patterns, specifically to better understand how dengue flows from urban to rural settings.

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PERFORMANCE OF THE DENGUE PLAQUE REDUCTION NEUTRALIZATION TEST (PRNT) IN PRIMARY AND SECONDARY DENGUE VIRUS (DENV) INFECTIONS ACROSS A SPECTRUM OF TEST CONDITIONS

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Dengue virus (DENV) infection has emerged as an important global health problem. The Plaque Reduction Neutralization Test (PRNT) is currently considered to be the "gold standard" to quantify circulating levels of anti-DENV neutralizing antibody (NAb) and, in seroepidemiologic studies, to identify seroconversions (infection rate) in a cohort over time. Validated NAb assays are required to support dengue vaccine development programs. Quantifying the development of NAb following administration of a vaccine candidate will likely be required as evidence of vaccine immunogenicity in support of a Biological License Application. Defining a NAb correlate of protection may be required to license a dengue vaccine in select populations. Many variations of the PRNT are currently in use and, despite noble efforts, neither the assay nor its components (e.g. control viruses, cell line, and other critical reagents) have been standardized. The authors used a well-characterized panel of acute and convalescent sera samples from children experiencing primary and secondary DENV infections to evaluate the performance of the dengue PRNT under a variety of performance conditions. Investigators varied cell type (BHK-21, LLC-MK2, and VERO), control virus passage (low passage, prototype, and prototype in tissue culture), and the use of complement across multiple assay runs of the same panel. Our findings indicate wide variation in PRNT results in response to varied testing conditions. Authors will review study methods, sera panel characterization, and resulting PRNT data. Dengue vaccine candidates are approaching efficacy trials. Defining the method to measure NAb represents a critical and time-sensitive scientific and regulatory requirement.

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SEROLOGICAL INVESTIGATIONS OF FLAVIVIRUS PREVALENCE IN KHAMMOUANE PROVINCE, LAO PDR, 2007-2008

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We report on the first largescale seroprevalence study of dengue (DEN) and Japanese encephalitis (JE) to be conducted in Lao PDR. The study was part of initial baseline public health impact assessments of the Nam Theun 2 hydroelectric dam construction project. Health surveys were performed from May 2007-Februrary 2008 during which serum specimens were collected from 3040 healthy individuals participating in the resettlement program of 14 villages (total surveyed population 4369). Hemagglutination inhibition assay using flavivirus antigens (DENV1, DENV3, and JEV) was performed on 1708 specimens at the National Center for Laboratory and Epidemiology in Vientiane, Laos. A total of 519 (30.4%) serum samples reacted positively to all flavivirus antigens tested, whereas 172 (10.1%) and 22 (1.3%) showed reactivity to JEV or DENV, respectively. These results indicate widespread transmission of flaviviruses within the watershed, and warrant improved surveillance and investigation to assess the clinical disease burden of flaviviruses in the area. Complementary entomological studies are ongoing during the current rainy season and will be discussed here.

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DO THE NONSTRUCTURAL PROTEINS 4A AND 4B OF DENGUE VIRUS CAUSE VESICLE FORMATION IN *AEDES AEGYPTI* CELLS?

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Flavivirus replication complexes have been shown to be sequestered in membrane-bound compartments in mammalian cells. We have evidence that similar sequestration of dsRNA in replication compartments occurs in mosquito cells and hypothesize that this allows dengue virus to evade RNA interference in infected mosquitoes. We have separated fractions of dengue infected mosquito cells by sucrose gradient flotation/sedimentation followed by analysis of gradient fractions via RT-PCR, infectivity assays, and strand-specific northern blots. Our results are consistent with the presence of membrane-bound dengue viral replication complexes in mosquito cells. To determine whether dengue virus encoded proteins are associated with the formation of vesicles originating from the endoplasmic reticulum membrane, as in mammalian cells, we have expressed nonstructural proteins 4a and 4b in an *Aedes aegypti* cell line. After verifying RNA and protein expression via northern and western blots, we will compare these cells to dengue virus infected and uninfected mosquito cells via sucrose gradient flotation/sedimentation, immunofluorescent microscopy, and electron microscopy. Results of these experiments will help elucidate the mechanisms by which dengue viruses evade the innate defense mechanisms of mosquito cells.

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DEFINING THE RELATIONSHIP BETWEEN DENGUE VIRUS TYPE-2 VIRAL GENETICS AND EPIDEMIC POTENTIAL IN PUERTO RICO, 1986-2007

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The mosquito-borne virus dengue (DENV) is endemic on the island of Puerto Rico, where co-circulation of 2 to all 4 serotypes occurs yearly. DENV-2 has been isolated from human sera specimens every year since 1986 by the passive surveillance system administered by the CDC Dengue Branch and was the dominant serotype identified in 10 of these years. We have sequenced the complete genomes of greater than 150 isolates collected from 1986-2007 and used this dataset to characterize the molecular evolution and phylogeny of DENV-2 in Puerto Rico during the study period. Temporal clustering was observed with isolates grouped into one major or one minor clade, each being further resolved into at least two distinct subclades. In order to identify the viral molecular determinants of DENV-2 transmission and therefore epidemic potential in Puerto Rico, representatives from each subclade were chosen for comparative examination of their infection and dissemination capabilities in field-collected *Aedes aegypti* mosquitoes. Genetic differences which we hypothesize to affect viral phenotype in *Ae. aegypti* were observed between the selected isolates in various genes encoding both the structural and non-structural proteins. Extinction and replacement events detected in our analyses suggest a mechanism by which DENV-2 is able to persist in Puerto Rico despite strong selective constraints imposed on the viral genome by the need to efficiently replicate in both *Ae. aegypti* and humans. The impact of observed sequence differences between major/minor clades and circulating/extinct subclades on the transmissibility of

these strains by *Ae. aegypti* and therefore the epidemiology of DENV-2 in Puerto Rico will be discussed.

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CHLOROQUINE IMPROVES PAIN AND WELL-BEING OF DENGUE PATIENTS BUT DOES NOT INFLUENCE THE PRESENCE OF FEVER AND DURATION OF THE DISEASE

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Dengue clinical manifestations range from a flu-like (dengue fever) to a severe, sometimes lethal, disease (dengue hemorrhagic fever/dengue shock syndrome [DHF/DSS]). The World Health Organization estimates that about 100 million people are infected by the dengue viruses every year, and as many as 500000 people develop DHF/DSS. Due to the lack of a dengue vaccine and the association of high viral load and DHF/DSS, we set up a double-blind study to administrate either chloroquine or placebo to patients suspected of having dengue disease. Patients were included in the study if they presented with fever and at least two other symptoms associated to dengue for less than 72 hours. Patients were excluded if they were pregnant, younger than 18-years old, or had either cardiac or neurologic disease. Once admitted on the study, they received 500mg of chloroquine *bid* for three days, and were re-evaluated a week later when they were informed if they had had dengue, and the information on the side effects and action of chloroquine on their disease were recorded. The study was conducted from mid-February to the first week of May of 2008, and 132 patients were included on the study, but three of them were excluded for lack of data on test results. Out of 129 patients, 63 received chloroquine, 32 were confirmed as dengue cases by at least two diagnostic tests (RT-PCR, MAC-ELISA, and NS1 antigen detection), and among the dengue patients, 19 received chloroquine. Side effects were reported by only two patients, and consisted of a transient blurred vision and loss of consciousness. Both patients were not confirmed as dengue patients. Among the dengue patients, there was no statistical difference in the duration of the disease, the intensity and days of fever. However, when asked if they had noticed anything different after taking the medication, 12 of them reported a great improvement in the intensity of pain and of their well-being. Interestingly, these patients declared that the symptoms returned after they had stopped taking the medication. Although only a small number of dengue patients were included on the study, about 60% of them reported an improvement of their symptoms while on medication, and the presence of side effects were unremarkable. Based on these results, we feel that chloroquine should be tested in a study containing with a higher number of dengue patients since if there is no drug to control dengue, at least chloroquine seems to alleviate the dengue symptoms.

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GENETIC ANALYSIS OF DENGUE VIRUSES COLLECTED FROM MOSQUITOES AND HUMANS DURING DENGUE CLUSTER INVESTIGATIONS IN THAILAND

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We conducted cluster investigations of dengue transmission in Kamphaeng Phet Thailand as part of a prospective study of primary age school children. An index case was identified from school absences and confirmed to be dengue by RT-PCR. Up to 25 contacts were enrolled living within a 100m radius of the child's home. Blood was taken from each of the contacts at day 0 and day 15. Mosquitoes were collected from the home of the index case and all homes within the catchment area on day 0. All clusters that contained at least one mosquito and one human that were positive for dengue by RT-PCR were included in this analysis to determine the genetic similarity between the human and mosquito viruses. From 2004 through 2007 we analyzed 15 clusters from all 4 serotypes (DENV-1, 8; DENV-2, 2; DENV-3, 1; and DENV-4, 4). There were a total of 10 mosquitoes and 14 human cases for DENV-1, 3 mosquitoes and 4 human cases for DENV-2 and DENV-3, and 7 mosquitoes and 10 human cases for DENV-4. The sequence alignment and phylogenetic analyses revealed that DENV from human and mosquitoes within each cluster were nearly identical (99.9-100 % E gene identity). Sequences of the same serotype collected at different times during the same year were distinct, revealing some change over time. We compared DENV-4 isolates from the city with DENV-4 isolates collected from the cluster investigation that occurred at the same time. Phylogenetic analysis revealed spatial structure indicating that viruses collected from clusters investigation were divergent from isolates collected from nearby districts but fell within the same clade if the isolates were collected from the same district within the same time period.

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EARLY CLINICAL AND LABORATORY MANIFESTATIONS OF DENGUE IN A PEDIATRIC COHORT STUDY IN MANAGUA, NICARAGUA

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Dengue is a major public health problem worldwide, with ~40 million cases annually. The wide range of clinical manifestations (undifferentiated febrile illness, classic dengue fever and dengue hemorrhagic fever/dengue shock syndrome [DHF/DSS]) complicates its early recognition, which is crucial for implementing appropriate treatment to prevent mortality of severe cases. A pediatric dengue cohort was established in Managua, Nicaragua in August 2004 and was used to characterize dengue symptoms and disease spectrum. Approximately 3,700 children (2-12 years old) who live in the catchment area of the Health Center Sócrates Flores Vivas (HCSFV) are followed, and detection of dengue cases occurs through enhanced passive surveillance via attendance to the health center. Acute and convalescent blood samples are obtained from subjects who met the WHO criteria for dengue or who present with undifferentiated febrile illness. Ninety-seven percent of febrile illnesses seek medical care at the HCSFV in the first 3 days after fever onset, and a convalescent sample is obtained from 95% of possible dengue cases. Dengue is confirmed by RT-PCR, virus isolation, and serologic assays. Through December 2007, 1,593 subjects completed the protocol for possible dengue cases, and 159 (10%) cases were confirmed as positive. Positive cases were classified as dengue fever (70%) and undifferentiated febrile illness (30%); 9 subjects developed DHF/DSS, all in 2007. The mean age of confirmed cases was 7.25 years old, and 53% were male. Viruses identified belonged to serotypes 1, 2 and 4 (25.6%, 73.7% and 0.8%, respectively), and a secondary immune response was observed in 55% of the cases. Multivariate analysis indicated that headache, rash, pharyngeal erythema/sore throat, no cough/no rhinorrhea, leukocytes $\leq$10,000/mm^3, and platelet count $\leq$200,000/mm^3 were significantly associated with symptomatic dengue at presentation ($p<0.001$). Rash was associated with primary immune response (OR 4.1, 95% CI 1.39-12.02), which is consistent with previous reports. During follow-up, the symptoms and signs associated significantly with dengue were lack of appetite, positive tourniquet test, petechiae, lymphadonepathy, and no rhinorrea ($p<0.05$), as evidenced by multivariate analysis. These clinical signs and laboratory

findings provide a useful tool for the early recognition of dengue in a pediatric population.

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TROPISM OF REPLICATING DENGUE VIRUS IN MICE AND HUMANS DEFINED BY VIRAL NONSTRUCTURAL PROTEIN 3-SPECIFIC IMMUNOHISTOCHEMISTRY

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Dengue virus (DENV) infections cause a spectrum of disease, ranging from self-limited dengue fever to fatal dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). Efforts to understand the pathogenesis of dengue disease have been hampered by problems in identifying the target cells infected by DENV in humans. All studies of human autopsies to date have identified infected cells based upon the presence of the envelope (E) and/or nonstructural 1 (NS1) proteins or positive-strand viral RNA, all of which circulate at high levels in the bloodstream during severe DENV infection and do not necessarily indicate replicating virus. Thus, the presence of these proteins or RNA in cells may result from endocytosis or phagocytosis rather than infection. To circumvent this problem, monoclonal antibodies were generated against the DENV2 NS3 protein, which is present only in infected cells. One monoclonal antibody, E1D8, was found to strongly react with NS3 in ELISA, flow cytometry, Western blot, and immunofluorescence. Additionally, E1D8 was able to detect infected cells in paraffin-embedded tissues from DENV-infected interferon receptor-deficient mice. Specifically, NS3-positive macrophages and dendritic cells in spleen and lymph node and hepatocytes in liver were identified by cell morphology, while double-immunofluorescence analysis identified infected myeloid cells in bone marrow. NS3-specific immunohistochemistry was then performed on a set of human autopsy tissues from fatal dengue cases in Ecuador. Infection was detected in mononuclear phagocytes in lymph node and spleen, alveolar macrophages in lung, and astrocytes and infiltrating peripheral blood cells in brain. Interestingly, DENV antigen was detected in hepatocytes, but not Kupffer cells, in liver in several cases. NS3 was also found in vascular endothelium of the spleen, but not other organs. Thus, NS3-specific immunostaining supports roles for infected phagocytes, hepatocytes, and vascular tissue in the pathogenesis of severe dengue.

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INCREASED PREVALENCE OF HYDROCELE, LYMPHATIC DILATATION AND TESTICULAR CALCIFICATION IN MEN WITH *MANSONELLA PERSTANS* INFECTION

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The filarial parasite, *Mansonella perstans* (Mp), is endemic in central and West Africa with a geographic distribution that overlaps those of *Wuchereria bancrofti* (Wb), *Loa loa*, and *Onchocerca volvulus*. Despite prevalences of Mp microfilaremia as high as 100% in some regions, little is known about the clinical manifestations of Mp infection. During an ultrasound survey of men coinfected with Mp and Wb in Sabougou, Mali, a surprisingly high prevalence of hydrocele was noted. Since Mp adults are generally found in serosal cavities, this raised the possibility that Mp infection was contributing to this pathology. To further investigate this association, ultrasound examinations were performed in 32 men with Mp monoinfection from the same villages as the original study and 23 normal controls living in Bamako. Concomitant Wb infection was excluded by history, nighttime thick smears for microfilariae and ELISA for circulating Wb antigen. The prevalence of hydrocele (≥ 10 ml) was 5/32 (16%) as compared to 35/63 (56%) in men coinfected with Wb and Mp and 0/23 (0%) in normal controls. Other abnormal findings included lymphatic dilatation in 31/32 (97%), and testicular and/or scrotal calcifications in 8/32 (25%), as compared to 57/63 (90%) and 16/63 (25%) in coinfected patients. Although mild lymphatic dilatation was also noted in 13/23 (56%) of the normal controls, scrotal calcifications were not seen in this group. Two patients with Mp monoinfection and extensive bilateral testicular microlithiasis underwent additional evaluation. Tuberculin testing and urine examination for eggs was negative in both patients. One patient, who complained of infertility, was found to have testicular atrophy and abnormal sperm. Interestingly, Mp microfilariae were detected in the hydrocele fluid of 2/15 patients with Mp infection who underwent hydrocelectomy and from whom fluid was examined at the time of the surgery. These findings suggest a heretofore unrecognized association between Mp infection and intra- and extra-testicular pathology.

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EFFECT OF ALBENDAZOLE AND IVERMECTIN DOSE ON *WUCHERERIA BANCROFTI* MICROFILARIAL CLEARANCE IN MALI: A RANDOMIZED, OPEN LABEL STUDY

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Albendazole and ivermectin are currently used in combination for annual mass treatment of *Wuchereria bancrofti* (Wb) infection in Africa. Although the drugs have been donated, the cost of such programs is very high and has proven to be a major impediment to the success of programs in countries with limited financial resources. Data from albendazole treatment of other filarial infections and one study comparing single to multi-dose DEC/albendazole in Wb infection suggest that increased dose and/or frequency of albendazole dosing may be more effective in maintaining microfilarial clearance. Furthermore, the optimal dose of ivermectin for suppression of Wb microfilaremia is greater than that being used in the mass treatment program. To determine the effect of increased dose and frequency of albendazole/ivermectin (A/I) treatment on microfilarial clearance, 42 Wb microfilaremic residents of an endemic area in Mali, were randomized to receive two doses of standard annual A/I therapy (400 mg/150 mcg/kg; n=22) or four doses of biannual increased dose A/I therapy (800 mg/400 mcg/kg; n=20). There were no differences between the two groups at baseline with respect to age, gender, Wb microfilarial levels or presence of worm nests on ultrasound. However, baseline circulating antigen (CAg) levels were increased in the annual treatment group (15,497 vs. 4,340 U/ml; p=0.03). Six months after the first dose of therapy, microfilarial clearance rates were comparable (16/22 (80%) in the annual standard dose group and 13/20 (65%) in the biannual high dose group). CAg levels decreased in all but 4 of the subjects (2 in each group) with a geometric mean decrease of 36% in the annual group and 57% in the biannual group (p=NS). Although these data do not support an effect of A/I dose on microfilarial clearance or CAg levels at 6 months post-treatment, the efficacy of both regimens at six months cloud the interpretation. The true impact of the increased dose and dosing frequency on the duration of microfilarial suppression requires assessment at 1 year (to be completed in July).

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ONCHOCERCIASIS AS A SIGNIFICANT CAUSE OF EPILEPSY: EVIDENCE FROM MODELLING THE RELATIONSHIP BETWEEN ONCHOCERCIASIS AND EPILEPSY IN 7 AFRICAN COUNTRIES

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The possible link between onchocerciasis and epilepsy is still a matter of debate. In the present study, we characterized quantitatively the relationship between the prevalence of onchocerciasis and that of epilepsy by including in a same model all the available data collected at a community level. Eight studies performed in West (Benin and Nigeria), Central (Cameroon and Central African Republic) and East Africa (Uganda, Tanzania and Burundi) met the inclusion criteria for analyses. As the methods used to diagnose *Onchocerca volvulus* infection varied between studies, the prevalences of onchocerciasis were standardized using specifically developed tools. The variation in epilepsy prevalence was then analyzed as a function of onchocerciasis endemicity using non-linear random effect regression modelling. A total of 91 communities (in which 79,270 individuals were screened for epilepsy) were included in the analysis. The prevalence of epilepsy ranged from 0 to 8.7% while that of onchocerciasis ranged from 5.2 to 100%. Variation in epilepsy prevalence is consistent with a positive exponential function of onchocerciasis prevalence, with epilepsy prevalence being increased by a 1.66 factor for each 10% increase in onchocerciasis prevalence. Using the current estimates of population living in meso- and hyperendemic areas for onchocerciasis (data provided by the African Programme for Onchocerciasis Control), we estimate that onchocerciasis-related epilepsy could represent 52,000 DALYs (Disability Adjusted Life Years) lost per year in Sub-Saharan Africa. These results give further evidence that onchocerciasis could represent a significant cause of epilepsy and that the burden of onchocerciasis might have to be re-estimated by taking into account this relationship.

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PROGRESS TOWARD ELIMINATION OF LYMPHATIC FILARIASIS IN THE EASTERN MEDITERRANEAN REGION

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Lymphatic filariasis (LF) is endemic in several Eastern Mediterranean Region (EMR) countries. LF in the EMR is caused by *Wuchereria bancrofti* and primarily transmitted by *Culex* in rural and semi-urban areas with *Anopheles* transmission in Southern Sudan. The estimated population at risk in the region is 12.6 million, representing 1% of the global LF burden. LF has been targeted for elimination under the GPELF umbrella in Egypt, Sudan and Yemen. Egypt (with an estimated population at risk of 2.7 million) initiated one of the first national LF elimination programs based on mass drug administration (MDA) with DEC and albendazole in 2000. The program used villages as implementation units (IUs) and included IUs with baseline infection rates ≥1%. The program maintained high coverage rates, reported (91%) by the government and (≥80%) by independent surveys. MDA was discontinued in 149 IUs (92.5% of the total) that met WHO stopping criteria after 5 rounds. The government is now providing MDA twice per year in 12 "hot spot" IUs that failed to meet stopping criteria after 5 rounds and in 17 IUs that have not yet completed 5 rounds in an attempt to quickly complete the "mop-up" phase of the program. Yemen's program targeted approximately ~110,000 people at risk with MDA with albendazole and ivermectin. Sentinel sites were surveyed for microfilaremia and for antigenemia in children in 2006 (after 5 rounds of MDA). Stopping criteria were met in all IUs except Socotra Island, where MDA is continuing. Sudan has a large LF problem and a complex political situation. Mapping activities have confirmed that LF is endemic in all 15 states in Northern Sudan (NS). A pilot MDA program (integrated with other disease control activities) is planned for two IUs in NS in 2008. A non-government organization is helping Southern Sudan authorities to complete mapping and initiate MDA in two states in 2008. Clinical LF cases have been reported in Djibouti, Oman, Pakistan, Saudi Arabia and Somalia. Additional work is needed to determine whether LF transmission occurs in these countries.

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A BLOOD AND FAECAL PARASITE SURVEY ON SATAWAL ISLAND, FEDERATED STATES OF MICRONESIA

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Satawal is a small isolated coral island located in the Federated States of Micronesia (FSM) with a population of approximately 500. A blood and faecal parasite survey conducted by a team from the FSM Department of Health and the School of Public Health and Tropical Medicine, James Cook University, Australia found a surprisingly high prevalence of parasites. Lymphatic filariasis is believed to be uncommon in FSM, but 34% of the Satawalese had a positive ICT filarial antigen test and 18% were microfilariae positive. Infection in males was approximately twice that of females, probably due to the differing exposure to infective mosquitoes at peak biting times. A mass chemotherapy program using Diethylcarbamazine and Albendazole has been introduced. Children from grades 1 to 8 were screened for intestinal parasites. *Ascaris lumbricoides* prevalence ranged from 100% to 69.2%, *Trichuris trichiura* from 28.6% to 7.7% and Hookworm from 1.5% to 7.7%. *Entamoeba coli, Entamoeba histolytica/dispar, Giardia lamblia* and other protozoa were also present. The intense transmission of intestinal parasites is almost certainly due to promiscuous defecation and measures must be taken to improve sanitation along with de-worming of children.

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HUMAN HELMINTH CO-INFECTION: A MULTI-LEVEL ANALYSIS OF SPATIAL, HOUSEHOLD AND FAMILIAL CLUSTERING OF POLYPARASITISM IN SOUTH-EASTERN BRAZIL

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Polyparasitism in humans is extremely common in tropical regions, and may have profound consequences for human health and well-being. Despite its wide-scale occurrence, the factors influencing patterns of polyparasitism within communities remain ill-defined. We have investigated the relative roles of exposure-related and host-dependent factors as sources of heterogeneity in the distribution of co-infection

with *Necator americanus* and *Schistosoma mansoni* in a region in the north-west of Minas Gerias State, south-eastern Brazil. Community-based parasitological, genealogical (pedigree), household and socio-economic data were combined with remotely-sensed environmental data using a geographical information system. Bayesian spatial statistics and hierarchical models were used to explore patterns of co-infection and assess the role of household and environmental risk factors in explaining spatial heterogeneity; a quantitative genetic analysis was then applied to evaluate evidence for shared genetic and non-genetic control of *N. americanus* and *S. mansoni* infection intensity. Results revealed considerable spatial, household and familial clustering of co-infection, with significant positive correlation of infection intensity between the two species. However, whilst a limited number of household and environmental risk factors could explain much of the spatial variability in the risk of co-infection, they could explain only an estimated 32% of variability between households. There was however no evidence for common genetic control of both infections (pleiotropy), although we did demonstrate a significant species-specific genetic component for *N. americanus* and *S. mansoni* infection intensity. Importantly, although there was some evidence of a household contribution to the relationship, much of the correlation between *N. americanus* and *S. mansoni* infection intensity remained unexplained. Taken together, these results emphasise the importance of shared exposure in the distribution of co-infection within communities, with much of the association between infections attributable to common exposures outside the household.

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VECTOR COMPETENCE OF *SIMULIUM OYAPOCKENSE* S.L. AND *S. INCRUSTATUM* FOR *ONCHOCERCA VOLVULUS*: IMPLICATIONS FOR IVERMECTIN-BASED CONTROL IN THE AMAZONIAN FOCUS OF HUMAN ONCHOCERCIASIS, A MULTI-VECTOR-HOST SYSTEM

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Although it is now well established that in the Amazonian onchocerciasis focus, straddling between Venezuela and Brazil, the main vectors in the highland (hyperendemic) and lowland (hypoendemic) areas, are respectively *Simulium guianense* s.l. and *S. oyapockense* s.l., investigation of the vectorial role of a third anthropophagic species, *S. incrustatum*, has remained inconclusive. Here we compare the vector competence of *Simulium incrustatum* with that of *S. oyapockense* s.l. by conducting, in the Venezuelan part of the focus, a series of fly feeding experiments designed to analyze their relative: a) microfilarial intakes when fed upon the same skin load; b) proportions of ingested microfilariae (mf) surviving damage inflicted by the cibarial armature (present in both species); c) survivorship to completion of the extrinsic incubation period; and d) infective (L3) larval outputs in surviving flies. Although the ability of *S. oyapockense* s.l. to ingest mf, for a given microfilaridermia, was markedly higher than that of *S. incrustatum*, the (density-dependent) proportions of those ingested mf that were damaged by the armature were also consistently higher, with the resulting output of L3 larvae being significantly lower in *S. oyapockense* s.l. than in *S. incrustatum*. The higher infective larval output in *S. incrustatum* may also be due to its greater survivorship after infection. These results, taken together, indicate that *S. incrustatum* plays a more important role in onchocerciasis transmission in the Amazonian focus than previously realized. We discuss the implications of our findings for the control and elimination of onchocerciasis with mass administration of ivermectin in this focus, where the three main anthropophagic species often co-occur.

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THE IMPACT OF A 10-YEAR CIVIL CONFLICT ON ONCHOCERCIASIS CONTROL IN SIERRA LEONE

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Onchocerciasis is widespread in Sierra Leone where it is endemic in all 12 provincial districts outside the Western Area. The Onchocerciasis Control Programme in West Africa (OCP), launched in 1975, initially covered a highly endemic area of the region including Burkina Faso, Mali, Cote d'Ivoire, Ghana, Togo and Benin. Operations were initially successful but hampered along the borders of the control zone by infected Simulium flies invading from neighboring countries such as Sierra Leone where blinding Onchocerciasis was also highly endemic. The OCP vector control area was therefore extended in 1989 to include north and central Sierra Leone in order to block the seasonal movement of the Simulium vectors. Larvicide treatment was maintained in Sierra Leone until 1996 when the conflict engulfed the entire country. Four years of efficient vector control drastically reduced the daily biting rate of the savanna black fly population from the 1988 pre-treatment level of 60 bites/person/day to 1 bite/person per day in 1994. Community microfilaria load decreased by over 90% over the same period. OCP was officially closed in December 2002 after virtually stopping the transmission of the disease in all the participating countries except Sierra Leone where operations were interrupted by a decade-long civil war. Surveys conducted after 2002 revealed that vector biting rates and community microfilaria load had reverted to pre-treatment levels in many communities. Since 2003, under the African Programme for Onchocerciasis Control (APOC), we have embarked on mass drug administration (MDA) using the Community Directed Treatment with Ivermectin (CDTI) strategy. With is method, communities plan activities with health workers and select volunteers who are trained to distribute Ivermectin in their own communities. CDTI has significantly reduced parasite prevalence and intensity in many communities.

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BURDEN OF LYMPHEDEMA DUE TO LYMPHATIC FILARIASIS - ORISSA STATE, INDIA

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India bears 40% of the world's burden of lymphatic filariasis (LF) and it is estimated that there are > 7 million people in India with lymphedema due to LF. Nevertheless, there are few LF-endemic areas that have performed a morbidity census documenting the extent of filarial disease, either lymphedema or hydrocele, in a population. In 2005, 40 local NGO's working in an LF-endemic district in Orissa State, conducted a house-to-house survey to assess the burden of lymphedema in a district with a population > 1.8 million. The survey enumerated the number of individuals with lymphedema in the district in preparation for the initiation of a district-wide lymphedema management program. Persons with lymphedema were asked a series of questions regarding stage of lymphedema and acute episodes of adenolymphangitis (ADLA). 17,036 (1%) persons reported lymphedema of the leg and 6,870 persons (0.4%) reported swelling in another body part (not-specified). The mean age was 49.3 years, range 0-99 years, and 57.6 % were male. The majority (97.9%) of persons with lymphedema or swelling resided in a rural area and 76.3% (16,410) reported a history of ADLA; mean 1.1 ADLA episodes/year, range 0-3. There were 993 (73.3 %) villages with $\geq$ 1 person with lymphedema; mean 22 persons/village, range 1-294, and 22.5% (4,839) of persons with lymphedema reported family members with lymphedema also. Women were more likely to report lymphedema of the leg (RR 8.34, 95% CI 7.52-9.26), yet men were more likely to report another body part with swelling (RR 3.45, 95% CI 3.26-3.65). Persons with lymphedema were more likely than persons without lymphedema to have had an ADLA episode in the past year (RR 1.76, 95% CI 1.70-

1.81). Older age was associated with an increased prevalence and higher stage of lymphedema ($p<0.0001$) and the probability of experiencing an ADLA episode within the past year increased with higher lymphedema stage ($p<0.009$). These data highlight the magnitude of lymphedema in LF-endemic areas and emphasize the need to better define the burden of chronic filarial disease in order to provide health services to those afflicted. Active lymphedema management programs which teach basic hygiene to decrease ADLA episodes and lymphedema progression should be prioritized and can be integrated with other chronic disease prevention programs at state and national levels.

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COMPLEX DYNAMICS IN PARASITE ECOLOGY AND CONTROL

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The current drive to eliminate neglected tropical diseases relies on a variety of lines of attack: mass drug administration, improvements in sanitation, health education, and vector control are a few. These approaches will each achieve different levels of impact on the infections and the diseases they are aimed against, at rates which depend on particulars of the host-parasite ecologies for each of these diseases. In the case of indirectly transmitted infections, such as lymphatic filariasis and schistosomiasis, these ecologies can be very complicated, involving the infection dynamics of several life-stages of the parasite within the definitive and intermediate host. Here, we investigate the consequences of parasite population dynamics, in particular the impact of regulatory feedback mechanisms, on the control of directly and indirectly transmitted helminth infections. We construct mathematical models of the relevant worm population ecologies, and investigate the effects of feedback density dependent mechanisms on 1) the existence of thresholds, e.g. the threshold vector biting rate, below which infection cannot be sustained; 2) the breakpoint parasite density, which implies the existence of a minimum parasite population below which extinction will result; 3) the rate at which the parasite level will change when it is perturbed from its initial level; and 4) the possible effect of all of these phenomena on multiple infection dynamics. The findings show that the actions and interactions between these density dependences at the population level can lead to complex population dynamics. We extract general practical principles from these results to highlight the importance of considering complex systems dynamics in the design of effective helminth control and even elimination programmes.

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ESTABLISHMENT OF MASS DRUG ADMINISTRATION PROGRAMME AND ITS ASSESSMENT: CASE STUDY OF MAFIA ARCHIPELAGO, TANZANIA

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The study aimed at defining the needs for the establishment of a Mass Drug Administration (MDA) programme for eliminating lymphatic filariasis in Tanzania. The strategy was to use a combination treatment of Mectizan and Albendazole to interrupt transmission and Mafia Island was used as pilot area for the process. Different methods and steps were followed up before the actual implementation of an MDA programme. Consultations were done with leaders at different levels as well as communities, on modalities to be followed up to successful, acceptable, replicable and sustainable programme. An assessment was carried out to determine levels of endemicity, knowledge and beliefs of communities regarding the disease transmission and cause. Patients suffering from clinical manifestations of lymphatic filariasis were interviewed and trained on how to take care of the affected parts. Patients with hydrocoeles were educated and convinced to go the district hospital for hydrocelectomy. Health officials, politicians and village health workers took the leading role in educating communities on the disease and the importance to take drugs and to participate in MDA process as a whole. Communities accepted the programme and participated in the MDA. Coverage in year one was 72.5% in year one in Mafia. The programme was successful in Mafia, and it was possible to initiate similar programmes in the other five districts of Coastal region in the year 2001. The programme established in Mafia was replicable to all other Coastal belt regions of Tanzania. Monitoring and evaluation was an important component of the programme. This was done after three and five rounds of treatments. Implication of the combination treatment was clearly seen it the studies area. People with clinical manifestations of LF reported improvement of the skin conditions and reduction in filarial attacks. Antigen levels and microfilaria loads decreased to around 10%, these results implies that the infection rates decreased but still there is a substantial levels of infection. Hence the study is suggesting more than five years of treatment and that more work should be done to increase coverage. Lastly, it will be important to think different when comparing reduction of infection in LF hyper and hypo endemic areas. It was noted that sustainable funding is crucial to programme success. The key element for success however that was distinctively noted was leadership.

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EVALUATION OF THE EFFECTS OF 3 ANNUAL ROUNDS OF MDA WITH IVERMECTIN AND ALBENDAZOLE ON BANCROFTIAN FILARIASIS IN MTWARA REGION, TANZANIA

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The primary objective of this study was to evaluate the effect of three years of Mass Drug Administration (MDA) on *Wuchereria bancrofti* infection in Mtwara region, Tanzania, using data from sentinel villages from two randomly selected districts. Overall, 559 people were sampled in both districts. Of these, 161 (29%) were females and 398 (71%) were males. The mean age for the participants was 31 years with a standard deviation (STD) of 16.4, with the minimum age being 5 and the maximum, 80 years. Five hundred and nineteen 519 (93%) of these individuals were amicrofilaremic and thus only 40 (7%) had microfilaria in their blood. The baseline mf prevalence in Newala was significantly higher at 14% while in Tandahimba it was 6.8% ($p = 0.000$). The geometric mean of mf count in Newala was 3.029/µl with a STD of 0.3485, while in Tandahimba, it was lower at 1.0/µl with STD of 0.714 ($p < 0.05$). The ICT card was successfully applied to 551 people, with 125 (23%) being ICT positive whilst 426 (77%) appeared negative. The ICT prevalence in Newala was again higher at 80/289 (28%), while in Tandahimba, it was 45/261 (17%). 551 participants were successfully tested for both ICT and microfilaraemia in the study; of these, 422(77%) tested negative for both ICT and mf, whereas 89 (16.1%) were ICT positive only and 4 (0.7%) were positive for mf only. 36 (6.5%) participants tested positive for both ICT and mf. After 3 rounds of MDA, mf prevalence in both districts declined significantly compared to the baseline data (2002), with the mf prevalence in Tandahimba district for year 2006 being 5.0%, while for Newala, 9.0%. Coverage rates between villages and regions were comparable, with average values ranging between 69%, 68% and 64% at the 2002, 2003 and 2004 MDAs respectively. Despite this, an important finding was that a slightly higher reduction in community mf prevalence from baseline levels was observed for those villages recording the higher drug coverages, irrespective of the study district. The total cost of the MDA for Mtwara was Tanzanian shillings 58,404,304/=being 80 shillings per individual treated and 50 shillings per person at risk. Most of the cost was attributed MDA(drug distribution) 24,149,780/= (41% of total cost).The results are

discussed in terms of the effectiveness of MDA programmes in eliminating LF infection in Mtwara, the effects of drug coverage on programme effectiveness, and the likely cost of MDA programmes for eliminating LF in Tanzania.

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THE IDENTIFICATION OF INFECTION IN ONCHOCERCIASIS ENDEMIC AREAS APPROACHING PARASITE ELIMINATION

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The identification of infection with *Onchocerca volvulus* has been traditionally addressed through a range of parasitological and clinical parameters, from the simple to the complicated. A number of onchocerciasis endemic areas are now approaching the complete elimination of the parasite where the definition of the actual presence of the infection has become increasingly difficult. Although rapid assessment techniques have been used successfully to implement mass drug administration programs, it is evident that these are less useful as the numbers of parasites present reach low levels in individuals and in the population as a whole. The existing traditional tests have sometimes produced confusing data in areas where there are very low loads of parasite, especially where only one type of test has been used. Data will be presented from investigations in Latin America and Africa that have shown that it is important to use combinations of the existing techniques and approaches when investigating the level of remaining infection. These combinations incorporate techniques such as post-treatment responses, patch tests, specific clinical presentations (ocular and dermal), standard parasite detection and the newer immunological/molecular approaches. Development of the optimal approaches to field assessment of infections requires a more detailed understanding of the parasite's biology and the disease's pathobiology. It is important to understand the biological implications and limitations of the information obtainable from these tests. This aspect will be discussed in light of recent information on the status of microfilarial and adult parasites (and onchocercal nodules), particularly in areas under intensive ivermectin (Mectizan®) treatment.

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TRANSMISSION HOTSPOTS AND FACTORS ASSOCIATED WITH CONTINUING TRANSMISSION OF LYMPHATIC FILARIASIS IN LEOGANE, HAITI

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Six rounds of mass drug administration (MDA) have been administered in Leogane, Haiti, an area hyperendemic for lymphatic filariasis (LF). Sentinel site surveys showed that the prevalence of microfilaremia was reduced from 16%, to < 1% suggesting that transmission had been reduced. We conducted a 30-cluster survey of 2-4 year old children to see if MDA prevented exposure to infection. Antigen and antifilarial antibody prevalence were 14.3% and 19.7%, respectively. We carried out follow up surveys in 6 villages that included previously selected clusters to assess risk factors related to continued LF transmission and to pinpoint and investigate hotspots of transmission. One hundred houses were mapped in each cluster using GPS-enabled personal digital assistants and then 30 houses and 10 alternates were randomly chosen for testing. All individuals in selected houses, ≥3 years of age, were asked to participate in a short survey about participation in MDA, history of residence in Leogane and general knowledge of LF. Survey teams returned to the houses at night to collect blood for antigenemia, microfilaremia and Bm14 antibody testing. Gravid traps were used to collect mosquitoes in each selected village. Antigen prevalence was highly variable between the 6 clusters, with the highest being 45.2% (Dampus) and the lowest being 3.0% (Corail Lemaire); overall antigen prevalence was 22.3%. Initial cluster surveys of 2-4 year old children were not well correlated with community antigen prevalence and transmission hotspots. Antigen prevalence among individuals who were noncompliant with the MDAs (25.8%) was significantly higher than in compliant individuals (15.9%) (p= 0.031). Bm14 antibody prevalence was higher in noncompliant individuals (52.8%) than in compliant individuals (44%). The highest percentage of non-compliant individuals (53.1%) was found in the village with the highest antigen prevalence and mosquito infection levels (Dampus). Thus, continuing transmission of LF seems to be linked to rates of systematic non-compliance.

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IDENTIFICATION OF MAJOR IMMUNOGLOBULIN E-BINDING PROTEINS IN TOTAL ADULT WORM EXTRACT OF *ONCHOCERCA VOLVULUS*

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Onchocerca volvulus, causes a chronic debilitating disease in humans known as *onchocerciasis* or river blindness. This disease is responsible for intolerable misery in some foci of the world as a result of its effects on the eyes, skin and kidney. *Onchocerca volvulus* infection in humans is characterised by elevated levels of serum immunoglobulin E (IgE) antibodies. This immunoglobulin isotype has been implicated in the pathogenesis of the severe skin manifestations associated with disease. To identify onchocercal allergens capable of inducing IgE antibody, adult worm extracts of *O. volvulus* were subjected to immunoblotting analyses using a hyper immune patient serum pool, and a normal African serum pool. About twelve proteins bands were detected as those that bind IgE, with 20 and 40 KDa being very prominent. Immunoblots on total *O. volvulus* extracts and *Loa loa* adult antigens probed with patient sera and controls also revealed allergens, but with 60, 67 and 100 KDa bands appearing as Onchocerca-specific. A comparative study was done with the related parasite bovine *O. ochengi* to investigate the possibility of it replacing the human parasite *O. volvulus* whose material is scarce and difficult to obtain from humans. This led to the identification of common and/or specific IgE binding proteins. Immunoblots on total *O. ochengi* extracts, probed with hyper-immune onchocerciasis patient serum pool, normal African serum pool, monkey loiasis serum and human schistosomiasis serum pool revealed a total of five protein bands those that bind IgE. Three of the five protein bands, with molecular weights 40, 60 and 67KDa were identical to those recognised in total *O. volvulus* extracts using hyper-immune patient sera. A 14.4 and 25KDa were uniquely *O. ochengi* specific, with the 25 KDa protein band uniquely detected on the strip that was treated with human onchocerciasis patient serum pool. These findings suggest the identified allergens should be excluded from an onchocerciasis / filariasis vaccine and that the proteins with relative molecular weights 60 KDa and 67 KDa may be useful as broad spectrum serodiagnostic molecules for human filariasis. They also show that the related bovine parasite *O. ochengi* antigens are poorly allergenic and may probable play a negligible role in the pathogenesis of bovine filariasis.

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DIFFERENCES IN EXPOSURE AND CHRONICITY IN HUMAN FILARIAL INFECTION LEADS TO VARIABLE GENE EXPRESSION PROFILES

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Distinct differences exist between patients with chronic, life-long filarial infection and those who acquire infection during temporary residency or travel (expatriates). Studies have shown that filarial-infected expatriates have both heightened immune responses and more overt clinical manifestations in comparison to endemic patients. To characterize the mechanisms underlying the differences in T cell responses in these patients, an analysis of global gene expression using human spotted microarrays (with 21,531 genes) was conducted on RNA from purified CD4+ and CD8+ cells from a group of microfilaremic endemic and expatriate patients infected with *Loa loa*. In the absence of antigen stimulation, the two groups expressed > 5,000 genes in common in both CD4+ and CD8+ cells. There were, however, a far greater number of genes differentially expressed by endemic patients for both CD4+ (133 genes vs 41 in expatriates) and CD8+ (146 genes vs 13) cells, many of which were associated with immune activation (e.g. MHC class II genes) and inhibition (e.g. IL-1R antagonist). For CD8+ cells, the differences in gene expression between the two groups were most striking, particularly in cell death genes (39 genes vs 2 in expatriates), which included members of the FAS network, and immune response genes (31 genes vs 11). Following *in vitro* stimulation with parasite antigen, only CD8+ cells from the endemic patients showed augmented gene expression above that seen for unstimulated cells. In contrast to findings for parasite antigen, stimulation with the nonparasite antigen streptolysin O induced expression of a large number of genes common to both patient groups, suggesting that the transcriptional differences between patients with chronic filarial infection and those with newly acquired filarial infection are primarily parasite-specific. These data suggest several pathways as being responsible for the distinctly different outcomes seen among filarial-infected patients with different levels of chronicity and exposure and imply an important role for CD8+ cells in these differences.

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CHRONIC HELMINTH INFECTION INCREASES THE THRESHOLD OF ACTIVATION FOR BASOPHILS AND MAST CELLS

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Although helminth infections elicit increased concentrations of IgE and Th2 cytokines, immune responses commonly observed with allergic diseases, populations infected with helminths have decreased rates of atopy. In this study, we tested the hypothesis that basophils and mast cells, effector cells of allergic responses that are stimulated through IgE, become harder to activate during chronic helminth infections. BALB/c mice were infected with *Litomosoides sigmodontis*, a filarial nematode of rodents. Peripheral blood and peritoneal cells from uninfected, acutely infected, and chronically infected mice were then stimulated with increasing concentrations of anti-IgE and evaluated for basophil and mast cell activation by multicolor flow cytometry. Basophil activation was determined by expression of surface CD200R and intracellular IL-4, and mast cell activation determined by surface expression of CD69. For basophils and preliminary data with mast cells, the amount of anti-IgE required for initial as well as maximal activation became greater over the course of infection, resulting in right-shifted activation curves consistent with decreased releasability. Importantly, over time basophils also became less responsive to IgE-independent activation by ionomycin, suggesting that decreases in releasability were not due to fluctuations in surface expression of IgE. These results indicate that basophils and mast cells become less responsive over the course of chronic helminth infection. While the mechanism for this change has not yet been determined, this finding may explain why individuals from areas with high prevalence of helminth infections have low rates of allergic disease.

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HISTAMINE DOES NOT PLAY A ROLE IN VACCINE-MEDIATED IMMUNITY AGAINST MURINE FILARIASIS

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The correlates of protective immunity in response to parasitic worm infections are not well understood. While numerous *in vitro* studies have demonstrated that helminth antigens can trigger release of histamine from basophils and mast cells, to date the role histamine plays in the immune response to helminth infections has not been investigated *in vivo*. We sought to determine the role histamine plays in the immune response to Litomosoides sigmodontis, a murine model of filariasis. A series of 3 vaccinations with irradiated larvae induces high titers of parasite-specific IgE and confers partial protection against a single challenge dose of infective-stage larvae. To determine if histamine is released in response to helminth infection, vaccinated and unvaccinated BALB/c mice were challenged with infective Litomosoides sigmodontis larvae, euthanized 30 minutes or 4 hours post-infection, and assayed for circulating histamine. All mice that had undergone primary and secondary infections had detectable levels of histamine 30 minutes after infection. To determine if histamine is a component of protective immunity, vaccinated mice were treated with either fexofenadine for H1 receptor blockade or cimetidine for H2 receptor blockade before and after infection. Mice were euthanized 8 weeks following challenge and analyzed for worm burden. Treatment with antihistamines did not alter the protective effects of vaccination. The finding that histamine release can be detected 30 minutes after infection of unvaccinated mice suggests that IgE-independent mechanisms of mast cell and/or basophil activation likely occur *in vivo*. This study also demonstrates that histamine likely does not play a role in vaccine-mediated protective immunity against helminths.

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CHANGES IN THE HIV-1 ENVELOPE GENE FROM NON-SUBTYPE B HIV-1 INFECTED CHILDREN IN KENYA

HIV-1 co-receptor usage plays a critical role for virus tropism and pathogenesis. Significant evolution in the HIV-1 envelope gene has been observed in some patients on effective HAART. This study was aimed at investigating whether HAART enhances co-receptor switch among HIV-1-infected children in Kenya. HIV-1 RNA was extracted from plasma samples obtained from 81 HIV-1-infected children between 2000 and 2007. Part of the envelope gene covering the C2V3 region was amplified by nested RT-PCR, sequenced either directly or after cloning, and analyzed genetically. Of 81 children 41 were on treatment. Of the 41 children, 28 used CCR5, 6 used CXCR4 as co-receptor, and 7 switched from using CCR5 to CXCR4 at the mean age of 7.3 years. The mean duration from treatment initiation to the time of the co-receptor switch was 2.6 years (range: 0.5-5.2 years). Of the 40 children without treatment, 29 used CCR5 and 8 used CXCR4 as co-receptors. Only 3 (9.4%) showed a switch in co-receptor usage from CCR5 to CXCR4 at the mean age of 9.7 years. On further analysis, 2 of 23 Long-Term-Non-Progressor (both without treatment) and 4 of 10 (40%) of Rapid-Progressor children showed co-receptor switch respectively. In conclusion, switching of co-receptor usage from CCR5 to CXCR4 among non-subtype B HIV-1-infected children did not seem to be enhanced by treatment, but by factors responsible for rapid disease progression.

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SEROEPIDEMIOLOGY OF DENGUE VIRUS INFECTION IN HIV-INFECTED CHILDREN IN COMPARISON TO HEALTHY CHILDREN

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Dengue infection is the most common arboviral infection in the world while HIV/AIDS is also one of the disease of global concern. The pathogenesis of both diseases is rather on the contrary and it is generally observed that denge hemorrhagic fever is uncommon in children with AIDS. This study was undertaken to study the seroprevalence of dengue virus infection in HIV-infected children in comparison to healthy children in Pediatric department, King Chulalongkorn Memorial Hospital. A cross-sectional serological and epidemiological study was conducted. Eighty-six HIV-infected children (age less than 15 years) and one hundred healthy children were enrolled. HIV-infected children were classified in categories by CDC 1994 criteria. Neutralizing antibodies to four dengue serotypes (DEN1, DEN2, DEN3 and DEN4) were measured by plaque reduction neutralization test (PRNT). From 1 year to 14 years and 11 months old children, 50 of 86 (58%) HIV-infected children and 65 of 100 (65%) healthy children (who had anti-HIV antibody negative by ELISA method) had positive neutralizing atibody against dengue virus by PRNT. There were no significant difference between these 2 groups (p>0.05). In HIV-infected children, a monotypic PRNT50 pattern was found in 26 children (30%) and multitypic pattern was found in 24 children (28%). Most children had neutralizing antibody against DE2. There were no significant difference between these 2 groups. In conclusion, HIV-infected children and healthy children had no different seroepidemiology of dengue virus infection.

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FIGHTING AGAINST HIV/AIDS AND MALARIA AMONG RURAL POPULATION OF HUYE IN SOUTHERN PROVINCE OF RWANDA

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Rwanda has been deeply upset by the 1994 genocide and is now in reconstruction process. According to DHS the impact of HIV/AIDS and malaria represent the single most menacing threat to achieve meaningful improvements in human development in Rwanda. HIV prevalence was estimated to 3% (DHS+2005). Malaria is amongst the top killer diseases in Rwanda and represents 40% of consultations in health facilities. Tuberculosis (TB) 6,046 cases were reported nationwide in 2003 and 60% of HIV patients are diagnosed with TB. With regard to HIV youth are at the greatest risk of contracting HIV and prevention is key to stemming the spread of HIV. Rwanda-VCP attempts to raise the knowledge of the local communities in southern province on HIV anad malaria through community education and mobilization. The present work aims to show impact of Rwanda-VCP strategies. Lesson learnt and encountered problems will also be addressed. Moreover, historical and current epidemiological aspects of HIV epidemic, Malaria and Tuberculosis in Rwanda will also be discussed. Rwanda-VCP strategies showed good results in regard to increase knowledge and reaching behavior change related to HIV/AIDS, malaria and TB issues among the rural population in South of Rwanda. The excellent way to address those issues is through the community based programs and effective communication.

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HIV/AIDS AND MALARIA CO-INFECTION IN DOUALA, THE ECONOMIC CAPITAL OF CAMEROON

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The objectives of the study were to determine the prevalence of HIV/AIDS and malaria co-infection and to determine and compare the prevalence of some parasitological, haematological and clinical parameters between co-infection and mono-infection of HIV/AIDS and malaria in the study population. A prospective cohort study on 684 adult hospital attendees in Douala, headquarters of the Littoral Province-Cameroon was undertaken. Information was collected on HIV/AIDS serostatus and malaria parasitaemia was assessed on blood smears by microscopy. Haemoglobin concentration was measured using the STANBIO STAT-Site® M^{Hgb} Test Kit (STANBIO Laboratory, Boerne, Texas, USA). CD_4 counts were obtained using the Partec CyFlow® counter (Partec Gmbh, Germany). The prevalence of malaria and HIV/AIDS co-infection was 29.4% (201). Geometric mean parasitaemia (parasites/µL blood) was statistically higher in co-infected (9 868) than in malaria (6 134) patients (F=3.44 p=0.018). Anaemia was more prevalent in co-infections (43.3%) compared with 36.8% and 20.4% for HIV/AIDS and malaria patients respectively (χ^2=12.38, p=0.006). Although the mean CD_4 count between co-infected and HIV/AIDS mono-infected patients was not significantly different (F=0.004, p= 1.000), more patients with dual infection had CD_4 counts corresponding to the chronic and advanced stages of HIV/AIDS infection. A total of 105 individuals were successfully followed up for 6 months and twelve deaths were recorded within this period, nine of which were co-infected patients. In conclusion, our results add to the existing pool of data from similar studies, showing that HIV and malaria co-infection have a significant effect on clinical outcome and therefore provide a basis for more elaborate studies with a larger sample size and follow-up of longer duration in the study region.

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EFFICACY AND TOLERABILITY OF THE BOTSWANA NATIONAL ANTIRETROVIRAL PROGRAM: FOUR-YEAR RETROSPECTIVE COHORT STUDY IN THE CENTRAL DISTRICT OF THE REPUBLIC OF BOTSWANA

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This study describes the treatment efficacy and tolerability of the Botswana National Antiretroviral Therapy Program (MASA) in Botswana's Central District. Inclusion criteria included a positive diagnosis of HIV, enrollment in MASA, and baseline data. Collected data included: patient history, clinical examination, and laboratory examination. Enrolled patients were treated according to a modified WHO HAART regimen. Additionally, enrolled patients were provided with TB treatment, TMP/SMX prophylaxis, and food supplements, if and when appropriate. Enrolled patients were required to present for follow-up at 2, 4, 12 months and every third month thereafter. Efficacy and tolerability were assessed by analyzing changes in information as recorded in the baseline data and in the data provided during each follow-up visit. Patient death and loss-to-follow-up was also indicated.

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A SURVEY OF STIS/HIV IN FEMALE PROSTITUTES IN NIGERIA

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A survey of Sexually Transmitted Infections (STIs/HIV) in female prostitutes in Lagos was investigated. A total number of 450 prostitutes were

randomly recruited for the study. Their ages ranged between 15 - 35 years with mean age of 23±4.They had pre- and post-test human immunodeficiency virus (HIV) counseling after giving their informed consent. Each participant completed a questionnaire in order to provide biographical data, sexual behaviour, knowledge of STI/HIV, knowledge and use of contraceptives, douching and average number of new sexual partners. Two cervical swabs (CS) and one high vaginal swab (HVS) were obtained from each participant. One CS was cultured immediately while the other was used to detect chlamydia antigen using Quick Vue chlamydia test kit. Wet preparations were made from the HVS for the detection of yeasts and trichomonads. Oral mucosa test (OMT) was used for HIV screening. Reactive participants were confirmed with Western blot technique. The blood samples were also screened for the presence of syphilis antibodies using rapid plasma regain kit. The positive samples were confirmed with *Treponema pallidum* heamagglutination test. Pathogens such as bacteria, fungi, and parasites were identified or isolated from 249 samples. *Candida* infections were more predominant (18.0%) followed by HIV-1 (17.1%), *Trichomonads* (12.8%), *Chlamydia* (3.1%), Syphilis (2.2%) and the least was *Gonorrhoea* (2.0%). The age distribution of STIs/HIV shows that age range 26-30 followed by age range 21-25 has the highest infection rate of 64.5% and 57.3% respectively. Knowledge and use of condom was high (88.4%). The difference in prevalence of STI in contraceptive users and those who do not use them are not statistically significant ($X^2_{0.05} = 0.2587$). The differences in prevalence of HIV infection between these categories and those who did not practice anal and oral sex was statistically significant ($X^2_{0.05}=0.000000$ and $X^2_{0.05}=0.0000012000$) respectively. There was a statistical difference between the CSW who practiced douching and those who did not in relation to HIV/STI ($X^2_{0.05}=0.0000002$). In conclusion, the prevalence of STIs/HIV amongst the population of prostitutes studied was high 55.3%. Studies concerning STI/HIV prevalence and its associated risk factors in Nigerian prostitutes should be continued in order to monitor trends in STI/HIV infections and the use of protective measures.

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MYCOBACTERIUM AVIUM COMPLEX ASSOCIATED PYOMYOSITIS, LYMPHADENITIS AND PERICARDIAL TAMPONADE IN HIV INFECTED PATIENT

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Mycobacterium avium complex (MAC) related pericardial effusion is very rare, but there are no case reports of MAC related pericardial effusion, pyomyositis and lymphadenitis occurring together. A 34 year old black female with HIV, AIDS, anemia, protein S deficiency, and seizure disorder presented with slowly growing neck mass and neck pain for 2 weeks was studied. She was taking efavirenz/emtricitabine/tenofovir (EET) for HIV infection for the last 16 months, azithromycin for MAC prophylaxis and trimethoprim/sulfamethoxazole for PCP prophylaxis. She was compliant with EET but not with azithromycin and trimethoprim/sulfamethoxazole. Her viral load was <75 copies/ml and CD4 count was 158 mm^3. She was prescribed amoxicillin/clavulanate and a biopsy of the neck mass was planned. During her follow up clinic visit the patient complained of shortness of breath and chest pain and was found to be hypotensive and tachycardic. She had clinical sign and symptoms suggestive of pericardial tamponade and 2D Echo confirmed hemodynamically significant moderate to large pericardial effusion. Immediate pericardial window was performed. CT scan of the abdomen and pelvis revealed focal low attenuation in the right psoas muscle suggestive of abscess, necrotic lymph nodes in the cardiophrenic angle and superior diaphragmatic space and necrotic nodal mass in the periceliac space. Aspirate of the neck abscess and pericardial fluid grew MAC. The patient was treated with amikacin, moxifloxacin, ethambutol, azithromycin and rifabutin with marked improvement. In conclusion, here are five previous case reports of MAC related pericardial effusion, but no case report of pericardial effusion, pyomyositis and lymphadenitis presenting together as was seen in this case. Most of the previous case reports of MAC related pyomyositis and skin abscess developed shortly after initiation of HAART as part of the immune reconstitution syndrome. Our patient however, was on HAART for 16 months before developing symptoms.

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DISPOSITION OF RURAL NIGERIAN ADOLESCENTS TOWARDS HIV TESTING AND DISCLOSURE

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HIV infection and related problems remain a major deterrent to the health of the youths in Nigeria largely because of the stigmatisation and misconceptions. Despite the enormous resources being spent on promoting HIV education and other preventive activities, a diagnosis of HIV is often viewed as a terminal illness, hence the unwillingness to undergo the screening and disclosure of results test. This study was carried out to assess the inclination of the Nigerian adolescents to HIV testing and disclosure of their results. This cross-sectional study involved consenting secondary school students enrolled using a stratified multistage random sampling technique at Shaki, a rural community in the South-West of Nigeria. Self-administered questionaires were used to obtain information of HIV testing and disclosure of result. Confidentiality was ensured. Descriptive analysis was done. Study participants comprised 1697 adolescents (851 males and 846 females), aged 10 to 19 years with mean age (±SD) of 15.6 years (±2.3). Over a third (33.5%) would decline voluntary HIV testing. Fear (54.0%5), surprise (60.2%), shame (46.8%), embarrassment (50.7%), and hopelessness (54.3%) were the various reactions they would express if positive to HIV testing. Concerning disclosure, 90.7% stated that they would disclose to someone if they tested positive to HIV. Among those to whom participants would disclose first were health workers (57.7%), parents (62.4%), teacher (15.7%) and boy or girl-friends (28.5%) while14.3% will not tell anyone. In the opinions of the participants, HIV was not considered to be a serious problem by 12.7%. In conclusion, this study revealed that though Nigerian adolescents were aware of the need but unwilling to know their HIV status and a significant proportion would not disclose to anyone. There is an urgent need to call attention to the necessity and benefits of HIV counselling and testing in the curriculum of secondary schools in Nigeria.

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USE OF HEALTHCARE FACILITIES AND THE IMPACT ON PREFERRED SOURCE OF TREATMENT FOR HIV/AIDS BY ADOLESCENTS IN NIGERIA

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Various factors have been shown to determine the choice of source of treatment especially in populations with cultures and beliefs that differ from the Western world. However attendance at orthodox clinics and hospitals by adolescents represent an important national medical issue as these habits is what is carried on into adult life. Few studies have characterized this issue among adolescents in African countries. The study was designed to determine the choice of use of orthodox health facilities by adolescents and relate this to their preferred source of treatment if they develop hiv/aids. The socioeconomic status, utilization of orthodox healthcare facilities and the preferred choice of treatment were retrieved from a semi structured questionnaire filled by adolescents in secondary schools in peri-urban areas in Oyo North, Nigeria. A total of 1697 adolescents, 851 males and 846 females participated in the study. The age range was 10years to 19years. 1010(60.3%) participants belonged to the lower socioeconomic class while 631(37.2%) belonged to the upper socioeconomic class. 69.2% (1174) claimed they visit a government hospital if sick while5.6% (95) will attend traditional or homeopathic

healers; 19.6% (333) will attend a faith based clinic or that run by an NGO. When asked at what point will they visit an health facility if they have symptoms of HIV/AIDS, 55.4% of those will visit a government hospital for treatment will see a doctor as soon as they realize it, 18.9% will wait for a month before presentation 20.7% will present after their treatment is abortive while 5.4 % will not visit a doctor. In conclusion, this study has shown that there may be a widespread reluctance to make use of government health facilities after diagnosis of HIV/AIDS among adolescents. There is need to raise awareness about the availability of treatments and other cares at hospitals in Nigeria.

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MONITORING THE VIROLOGICAL RESPONSE TO ANTIRETROVIRAL TREATMENT IN ADULT HIV/AIDS PATIENTS IN TANGA, TANZANIA

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Monitoring virological responses to antiretroviral (ARV) treatment by means of HIV-RNA levels is a reliable but expensive procedure, which is therefore not routinely used in resource-limited treatment facilities in Africa. An alternative cheaper method has been proposed: the ELISA-based p24 antigen assay. The aim of this study was to compare results of the conventional HIV-RNA assay and the alternative p24 antigen assay for the assessment of responses to ARV treatment in a group of adult HIV/AIDS patients in Tanga, north-eastern Tanzania. 40 adult patients with HIV/AIDS, who met the criteria for initiating ARV treatment as part of the government treatment scheme in Tanzania, were recruited for the study and followed up during four months of first-line treatment with Triomune® (Stavudine + Lamivudine + Nevirapine). Levels of HIV-RNA and p24 antigen were both analysed in plasma samples collected at baseline and after two and four months of treatment, respectively. Compliance questionnaires were completed and Nevirapine concentrations measured with an ELISA-based assay. RVs were accepted and taken well by most patients, according to the compliance questionnaires and measurable Nevirapine levels in 89% of the samples from patients who completed the follow-up period. The ARV treatment showed a strong effect during the first two months of patient follow-up (N = 40). Median log HIV-RNA levels were reduced from 5.3 copies/mL to 2.0 copies/mL (P<0.001), while median p24 antigen levels were reduced from 39.7 pg/mL to 0.5 pg/ml (P<0.001). Importantly, HIV-RNA and p24 levels both remained low during the following additional two months of treatment; however, only about half of the patients (N = 23) completed four months of follow-up. In conclusion, short-term ARV treatment was found to be highly effective as assessed virologically both by the conventional HIV-RNA assay and the alternative cheaper p24 antigen assay. The p24 assay may serve as a useful tool for patient assessment and as an indicator of treatment adherence in resource-limited settings.

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COLONIZATION WITH CEFOTAXIM RESISTANT *ENTEROBACTER* SPP. AND *KLEBSIELLA* SPP. IN HIV-POSITIVE CAMBODIAN CHILDREN DECREASES WITH IMMUNE RECONSTITUTION AFTER HAART

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HIV-positive children are exposed to multiple antimicrobials. About 30% of them receive HAART due to low CD4 count, one half of them also antiTB drugs and due to several episodes of opportunistic infections they also receive multiple courses of antimicrobials mainly for treatment of respiratory tract infections, candidiasis and herpes infections. The aim of this short communication is to assess the impact of HAART and/or previous exposure to antibiotics on colonization of cefotaxim resistant *Enterobacter* spp. and *Klebsiella* spp. in HIV-positive children. One hundred and two (102) HIV-positive children from Phnom Penh in Cambodia treated with HAART for 52 months were cultured by throat and nose swabs in 3 months intervals (6 months in 2007). Isolates were transported in Amyes agar and liquid Sabouraud's dextrose agar (4 per patient) within 24 hours after obtaining the culture to the National Reference Laboratory for Antimicrobial Resistance in Slovak Republic by air. Antimicrobial susceptibility testing was performed according to NCLLS. Consumption of each antibiotic was recorded in the chart of particular children. Prior exposure to antiobiotic was defined as receipt of antibiotic 14 days prior to the day of positive culture. On baseline until 6 months of HAART all *Enterobacter* spp. and *Klebsiella* spp. isolates were resistant to cefotaxim (CTAX), then after immune recovery in month 9 resistance rates dropped to 11%-0% but subsequently increased to 10-25%. After 3 years of anti-HIV treatment no *Enterobacter* spp. and *Klebsiella* spp. isolates resistant to cefotaxim appeared at all. From 91 strains, 21 (23.1%) were resistant to cefotaxim during entire evaluated period, 90% were susceptible to gentamicin, 29% to cotrimoxazol and 95% to ciprofloxacin. Colonization with cefotaxim resistant *Klebsiella* spp. and *Enterobacter* spp. was not significantly associated with prior cephalosporin but cotrimoxazole use (P<0.05). In addition HIV-positive children on HAART who received any antibiotic were significantly more colonized with cotrimoxazole resistant *E. coli* (P<0.01) in comparison to children not receiving any antibiotic prior to colonization. In conclusion, reconstitution of immune status and increase of CD4 T-cells due to HAART decreased colonization with cefotaxim resistant *Enterobacter* spp. and *Klebsiella* spp. isolates in HIV-positive children.

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LDLIP3 ENCODES A SECRETED LIPASE IN THE HUMAN PATHOGEN *LEISHMANIA DONOVANI*

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Lipases are ubiquitous enzymes that hydrolyze the ester linkages of fats to form glycerol and fatty acids. These enzymes are involved in biological activities ranging from antigenic variation and cell signaling to nutrient acquisition and have been implicated as virulence factors in some pathogens. We hypothesize that the lipase activity released by *Leishmania* may play an important role in the biology of these primitive parasites. Culture supernatants of *Leishmania* were incubated with various 4-methylumbyliferyl fatty acids. Results of these assays identified lipase activities from *in vitro* culture supernatants of *Leishmania*. Subsequently, a PCR-based strategy identified a candidate lipase gene from *Leishmania*. The *L. major* genome database revealed 3 putative secretory lipases. The homologue of one of these, designated LIP3, was cloned. Sequence analysis of LIP3 revealed an ORF of 927bp and a deduced protein of 308aa with a predicted molecular mass of 33.0kDa. Further analysis showed a

putative 24aa signal peptide and the absence of an anchor motif, both consistent with a secretory molecule. Moreover, a conserved serine-lipase active site was identified. Southern analysis revealed the presence of more than one copy of this gene in the *Leishmania* genome. RT-PCR revealed that this gene is expressed in both promastigotes and amastigotes. To further characterize this lipase, an epitope-tagged construct was episomally expressed. Western analysis revealed that the expressed protein was secreted by transfected *Leishmania*. Lipase activity of the expressed protein was determined using 4-methylumbyliferyl fatty acid substrates on immunopreciptates from transfectant culture supernatants. Taken together, our data supports the hypothesis that lipase activity encoded by LIP3 is secreted by *Leishmania*. Further characterization of this gene and protein will lead to a better understanding of the role of lipase within the biology of the important group of human pathogens.

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SECRETED PROTEINS OF *LEISHMANIA CHAGASI INFANTUM*-PROGRESSING TOWARDS A SUBUNIT ANTI PARASITE VACCINE

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The fatal disease visceral leishmaniasis (VL) is caused by the protozoan *Leishmania chagasi infantum* (*Lci*), an obligate intracellular parasite of macrophages. Despite its prevalence in the underserved regions of the world, treatment of VL is problematic due to expense, and the need for prolonged and toxic treatment with drugs to which parasite isolates are developing resistance. This has led to an increased interest in vaccine development. Spontaneous or drug-induced recovery from VL leads to a Type1 cellular immune response and immunity, which protects against re-infection, providing the rationale for vaccine development. Despite numerous efforts, there is no safe, avirulent vaccine against VL. The excreted/secreted (ES) proteins of *Lci* likely constitute the first parasite-derived molecules that are perceived by the host immune system and may be critical virulence factors that suppress or modulate the host immune response. Furthermore, *L. infantum* promastigote culture filtrates elicit strong immunity and protection against *Leishmania* spp. in BALB/c mouse and dog models of leishmaniasis. Therefore, we analyzed the annotated *Lci* genome to predict a set of potential ES proteins based on sequence characteristics. First, proteins predicted to enter the secretory pathway and transit through the ER were identified with the TargetP algorithm. These were then analyzed for a signal peptide using SignalP, and for the absence of transmembrane domains using TMHMM and PHOBIUS programs. Because many *Leishmania* spp. surface proteins possess a glycan phosphatidyl inositol (GPI) membrane anchor and these would also traffic through the secretory pathway, we checked for absence of a GPI anchor addition site using the algorithms big-PI Predictor and GPI-SOM. The resultant set consists of 250 candidates from *Lci* that are predicted to enter the secretory pathway, have signal peptides and lack transmembrane domains and GPI anchor sites. Of these, 162 are hypothetical proteins and 88 are or homologues of known proteins. This approach was validated by the finding a number of proteins already reported as *Leishmania* spp. secreted proteins (e.g., acid phosphatase, chitinase and P1/S1 nuclease). Based on the annotated identities, 17 candidates were selected for further expression, antibody generation and localization studies. These and other ES proteins constitute candidate virulence factors that could be studied as potential vaccine antigens.

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SUPPRESSION OF HOST MACROPHAGE TRANSCRIPTIONAL RESPONSES BY *LEISHMANIA MEXICANA*

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The protozoan pathogen, *Leishmania mexicana* causes chronic, non-healing lesions in humans and C57BL/6 mice. In order to formulate a broad transcriptional profile of macrophages infected with *L. mexicana*, we performed microarray analysis on C57BL/6 bone marrow-derived macrophages (BMM) infected with unopsonized *L. mexicana* promastigotes over a 24 hour time course. The transcriptional profile of cells infected with *L. mexicana* was almost indistinguishable from that of control, uninfected cells. In order to determine if this "silent" infection was the result of active suppression on the part of *L. mexicana*, we treated macrophages with heat-killed *L. mexicana* promastigotes over the same time course. Macrophages treated with heat-killed parasites were activated, producing a transcriptional signature that included upregulation of many interferon-responsive genes. In order to identify the virulence factor in *L. mexicana* that is responsible for the suppression of macrophage responses, we infected BMM with *L. mexicana* cysteine protease B (CPB) knock outs and *L. mexicana* cysteine protease B/cysteine protease A (CPA) double knock outs (obtained from J. Mottram). Previous studies have shown that *L. mexicana* CPB plays an important role in the inhibition of Th1 immunity in C57BL/6 mice and that CPB expression in *L. mexicana* amastigotes results in inhibition of IL12. However, the role of CPB and CPA in *L. mexicana* promastigotes has yet to be elucidated. We found that infection with *L. mexicana* deficient in CPB or *L. mexicana* deficient in both CPB and CPA resulted in upregulation of interferon-responsive genes, characteristic of an inflammatory response. These data suggest that *L. mexicana* promastigotes actively suppresses host transcriptional responses by a mechanism that is at least partially dependent on cysteine protease B and cysteine protease A.

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METACYCLOGENESIS ALTERS RECEPTOR-MEDIATED UPTAKE OF *LEISHMANIA CHAGASI* PROMASTIGOTES BY HUMAN MONOCYTE-DERIVED MACROPHAGES

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The protozoan parasite *Leishmania chagasi* is the causative agent of visceral leishmaniasis in the New World. The promastigote form of the parasite (PM) develops from a logarithmic-like stage to an infectious metacyclic form inside the gut of the sand fly vector. During a sand fly blood meal, metacyclic PMs are inoculated into mammalian skin, where they are ingested by macrophages and other phagocytic cells. Following internalization into macrophages, normal phagolysosome maturation is delayed, permitting the parasite to transform into the stress-resistant amastigote stage. Several studies have documented this fusion delay, and others have identified an array of receptors for entry into macrophages, including the mannose receptor (MR) and the third complement receptor (CR3). However, it is unknown whether uptake via different receptors leads to intracellular killing, and furthermore, whether the routing pathways differ between different PM forms. We hypothesized that log stage and metacyclic forms of Leishmania initiate entry through different receptors, and thus, may be trafficked through subsequent intracellular pathways that influence their survival. Using confocal microscopy, we detected MR or CR3 ligation during phagocytosis of *L. chagasi* PMs by human monocyte-derived macrophages. Log PMs co-localized with MR as early as 2 minutes after initiating infection, and peaked at 30 minutes, after which, intracellular parasites began to lose MR staining. During this peak, 50% of PMs from log culture co-localized with MR, where as only 8.6% of purified metacyclics co-localized with MR ($p < 0.001$). Heat killed PMs were taken up more avidly than either log or metacyclic PMs, and after 30 minutes, 42% co-localized with MR. The observed MR co-localization contrasted from those of CR3, which were often associated with metacyclics immediately prior to internalization. These data suggest that avirulent log or heat-killed *L. chagasi* PMs are taken up by the MR, but the highly virulent metacyclic form uses other receptors for macrophage entry. In addition to our growing knowledge of Leishmania phagocytosis via quiescent pattern recognition receptors, we now see that metacyclic promastigotes are capable of selectively bypassing some macrophage entry pathways.

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FUNCTIONAL VARIATIONS IN CANDIDATE GENES FROM INNATE IMMUNE RESPONSE (MCP-1 AND MBL2 GENES) ARE ASSOCIATED WITH MUCOSAL LEISHMANIASIS OUTCOME IN CORTE DE PEDRA, BAHIA, BRAZIL

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T helper (Th1) cell type 1-mediated immunity is essential for human defense against Leishmania infection, but an exaggerated Th1 immune response is associated with mucosal leishmaniasis (ML). ML follows cutaneous leishmaniasis (CL) caused by *Leishmania (L.) braziliensis* infection. *Our hypothesis is that a functional variation in genes that influences inflammatory response is associated to ML outcome.* The study followed two designs: a case-control (60 subjects from the groups: ML, CL, Neighborhood control without history of disease - ND control and subjects with a positive delayed-type intradermal response to leishmania antigen - DTH+); and a family (67 families selected from ML index-cases). Two candidate genes were studied: 1) Mannose-binding lectin (*MBL*) and 2) Chemokine monocyte chemoattractant protein-1 (MCP-1). We genotyped 6 polymorphisms in MBL2 gene: three structural variants in exon 1 at codons 52 (CGT to TGT), 54 (GGC to GAC) and 57 (GGA to GAA), corresponding to amino acid changes, resulting in low levels of serum MBL (termed as O allele while the wild is A allele); two SNPs at *-550 G/C* termed *H/L* and at *-221C/G* termed as *X/Y* (also affect the serum MBL levels); another SNP located at position +4 *C/T* in exon 1 (termed *P/Q* - the haplotype LYA into LYPA and LYPQ that corresponds respectively to intermediate and high MBL expression); and from MCP-1 gene we evaluated one polymorphism at position -2518 G/A. Genotype frequencies were compared among the groups using logistic regression and family-based association test (FBAT). An association was noted between the SNP *-221X/Y* of the *MBL*2 gene and ML (P(Pc) = 0.003(0.024); OR = 0.48 [0.28 - 0.82] suggesting that the *-221Y* allele confer low risk to develop ML. The analysis of the MCP1 -2518 G/A SNP revealed a an association of the G allele with ML versus NC (0.045; OR 1.78 [1.01-3.14]. The FBAT confirmed the association of G allele with ML under recessive model (z=2.69, p=0.007). Higher levels of MCP-1 were measured in plasma and macrophage culture supernatants from GG compared to AA individuals. This study demonstrated associations between two genes polymorphisms encoding proteins from the innate immune response with ML, suggesting that they might influence leishmaniasis clinical outcome.

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A NEW CLASS OF OXABOROLE-BASED POTENT ANTITRYPANOSOMAL AGENTS: PROBING THE EFFECT OF DIFFERENT CENTRAL LINKAGE GROUPS ON *TRYPANOSOMA BRUCEI* GROWTH INHIBITION

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Human African trypanosomiasis (HAT), or sleeping sickness, is caused by protozoal parasite *Trypanosoma brucei*. HAT affects about 60 million people around the world and represents a significant health threat. Though suramin, pentamidine, melarsoprol and eflornithine are the current treatment for early-stage or late-stage HAT, it is urgent and challenging to discover new anti-trypanosomal agents due to the emergence of resistance, and poor safety profile or high cost of the existing drugs. The series of oxaborole derivatives reported here represents a new class of potent antitrypanosomal agents that showed *in vitro T. brucei* growth inhibition with IC_{50} values as low as 0.02 μM and *in vivo* efficacy in mice infection model. The effect on *in vitro* potency of different central linkage groups was investigated by comparing an array of functional groups. The thioether, sulfoxide and sulfone linkage groups showed comparable potency although they have different bond angles and hydrogen bond forming capability, while introduction of amide or sulfonamide linkage group significantly increased the potency. We present here SAR data of different linkage groups and their phamacophore modeling study. The early SAR of the *in vivo* efficacious compound will also be discussed.

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DEVELOPMENT OF AN ALAMAR BLUE 384 WELL WHOLE CELL VIABILITY ASSAY FOR HTS OF *TRYPANOSOMA BRUCEI BRUCEI* BS 427 CELLS

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African human trypanosomiasis (HAT) is caused by two species of trypanosomes; *trypanosoma brucei rhodiense* and *trypanosoma brucei gambiense*. During the second stage of infection severe neurological symptoms present, progressing to a somnolescent state and is fatal if untreated. The disease occurs in 36 countries in sub Saharan Africa and there is an incidence of around 500000-700000 cases on an annual basis reported by WHO. Most drugs are not effective against both trypanosome species and disease stages, have expensive and difficult treatment regimes and all have some associated cytotoxicity. There is an urgent need for new compounds to be fed in to the drug development pipeline. One approach to drug discovery is HTS of diverse compound collections for the identification of active hits. For the application of HTS it would be of benefit to employ 384 well assays there are currently no reports in the literature for 384 well whole cell screening of *brucei* trypanosome species. We have developed a 384 well assay for the estimation of viability of *T.b.brucei* BS427 whole cells using the Alamar Blue viability assay, which has been previously reported for *t.b.brucei* species in 96 well format. The assay was developed to HTS standards by measuring statistical parameters including Z'-prime, %CV and signal to background ratio. Optimisation of the assay incorporated such parameters as cell concentration, DMSO solvent sensitivity and compound dilution media. The cell optimised cell inoculum concentration was based upon comparisons of assay reproducibility from cell inocula ranging down from the maximal in plate/flask concentration ($3x10^6$ cells/mL) following 72 hours growth. The IC_{50} of the reference compounds Diminazene Aceturate and Pentamidine were compared to those reported in the literature and were found to be comparable. The development and optimisation of the HTS assay will be described in detail.

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TOWARDS THE IDENTIFICATION OF PROTEINS INVOLVED IN TRANSCRIPTION OF THE U2 AND U4 SMALL NUCLEAR RNA GENES IN *LEISHMANIA*

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Leishmania is a trypanosomatid parasite that produces leishmaniasis in humans. Trypansosomatids possess unique mechanisms of gene expression, like polycistronic transcription and trans-splicing. Our group

is interested in the study of transcription by RNA polymerase III (Pol III), which transcribes small essential RNAs, such as tRNA, 5S rRNA and small nuclear RNAs (snRNAs). Little is known about Pol III transcription in trypansosomatids. A few snRNA-gene promoters have been characterized in *Trypanosoma brucei* and *Leptomonas*. These promoters consist of two conserved sequences (box A and box B) contained in a tRNA or tRNA-like gene located upstream of the snRNA gene and in opposite orientation. In most cases, the first 10 bases of the snRNA gene are also required to achieve an optimal level of expression. Similarly to other trypanosomatids, our analysis of the sequences upstream of the U2 and U4 snRNA genes in *L. major* and *L. mexicana* led to the identification of divergently oriented tRNA-like genes. To explore the binding of nuclear proteins to these putative promoters, we performed electrophoretic mobility shift assays, using fragments that contain the entire tRNA-like sequence as a probe. Our results showed the presence of several retarded bands for both genes. Competition experiments indicated that at least one of the observed bands is specific. We are currently testing other competitors to explore the possibility that different types of Pol III promoters might interact with the same nuclear proteins. The retarded DNA-protein complexes will be purified from the gels to try to identify the proteins that bind to the putative promoters.

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POLYMERASE CHAIN REACTION (PCR) FOR DIAGNOSIS ON VERTICAL TRANSMISSION OF SECOND GENERATION OF *TRYPANOSOMA CRUZI* IN WISTAR RATS

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Trypanosoma cruzi is the etiological agent of Chagas`disease and can be transmitted during the acute and chronic stages by an infected mother via placenta and during lactation. Congenital Chagas disease of second generation has been reported in Chile. This study attempted to quantify this form of transmission of second generation of *T. cruzi* in offspring from "healthy" mothers rats, were born from rats initially intradermally inoculated with metaciclic tripomastigotes of *T. cruzi.* The infection was evaluated 30 days after parturition. Direct parasitological examination of blood samples and blood culture carried out on 42 offspring did not reveal patent parasitemias; the xenodiagnosis revealed a positivity of 19.05% (8/42). Serological tests with IIF and ELISA showed anti-*T. cruzi* antibodies in 33.3 and 35.7 % of offspring. Histopathology of the heart did not showed parasitic persistence in cardiac muscle fibers, mild myocarditis and myositis with signs of chronicity; discrete inflammatory infiltrate of variable intensity, without parasitism. Immunohistochemical examination (PAP and IIF) showed the presence of abundant antigen deposits in section of heart and skeletal muscle, revealing the presence of tripomastigotes on the tissue. The PCR applied to sections of cardiac and skeletal muscle of the offspring, displayed amplified of rest of DNA of *T. cruzi* in a 80 % (16/20). The PCR detection of *T. cruzi* DNA in cardiac and skeletal muscle, showed higher sensitivity respect the xenodiagnosis test ($p<0.001$). These results suggest that vertical *T. cruzi* transmission of second generation occur in the experimental model Wistar rats.

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PRODUCTION OF CYTOKINES IN FETUSES OF INFECTED MICE NMRI WITH *TRYPANOSOSMA CRUZI*

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The congenital infection with *Trypanosoma cruzi* is a public health problem that allows the uncontrolled transmission of parasites from one generation to another. The present study examined the fetuses of NMRI mice infected with $22x10^3$ trypomastigotes metacyclic the strain M/HOM/BRA/53/Y of *T. cruzi* and pregnant during the acute phase of infection. The infection showed the highest levels of parasitemia in mice with 30 days of infection and 20 days of gestation compared with mice infected virgins. The fetuses of mice infected and uninfected (control group) were obtained at the end of gestation. Infection by *T. cruzi* in pregnant mice affected intrauterine development in three of the fetuses 3 (15%), which had congenital anomalies and structural morphological muscle, two of these fetuses were mothers with higher levels of parasitemia patent. The fetuses of mothers infected showed in turn, weight loss, stunted growth and reduction in the number of them, from 9 to 6, compared with 14 healthy fetuses of pregnant mice. The detection of INF-γ, IL-4 and IL-10 was carried out in sections 7 µm in the placenta, skeletal muscle and heart fetal using anti-INF-γ, anti-IL-4 anti-IL - 10, IgG PE conjugated and anti-CD4 FITC conjugated. In these fetal tissue cytokines IL-10 and IFN-γ were detected, and lymphocytes CD4 + and CD4- producing IFN-γ, since they are the source of this cytokine production at any stage of infection. The histopathologic study conducted in this fetal tissue revealed the presence of abundant inflammatory infiltrate in the absence of parasitism tissue. These findings suggest that the fetus is capable of generating an immune response own front to antigens transmitted by her mother, which induces the secretion of cytokines that act in synergy with the maternal antibodies confer a state of protection against infection and transmission of the parasite depends on factors specific to each mother, which may modify its ability to control such transmission to placental or systemic level.

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IMPROVING DRUG ADHERENCE AMONG VIVAX MALARIA PATIENTS IN NORTHERN THAILAND

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Drug adherence is very important in vivax malaria treatments. In northern Thailand, 76.2% of vivax malaria patients failed to complete the 14-day course of medication. This study emphasized the need to educate vivax malaria patients to comply with the treatment. The quasi-experimental research was conducted in 12 malaria clinics (MCs) in two districts in Mae Hog Son Province. The Information, Education, and Communication (IEC) program, consisting medication instructions, poster displays, flip charts, pamphlets, and newly designed drug bags, was administered to the intervention group. The control group only received a usual routine for malaria treatment. The data collection to elicit information from the patients was quantitative and qualitative methods. We compared pre-intervention questionnaire responses to post-intervention responses at 14 days after attending MCs using the same questionnaire as pre-intervention. Data were analyzed using STATA version 8.0 (STATA Inc., College Station, TX). Chi-square test/Fisher extract test, Student's t-test, and paired t-test were used to describe the data. After receiving the IEC program, 71.1% of the patients in the intervention group completed their prescribed treatments while 29.9% in the control group adhered to it. The mean scores of knowledge, perception of malaria treatment, self-efficacy in drug taking, and having satisfaction of health services