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PREVALANCE OF LYMPHATIC FILARIASIS IN AMERICAN SAMOA AFTER THREE YEARS OF IMPROVED SOCIAL MOBILIZATION AND MASS DRUG ADMINISTRATION

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As a member of the Pacific Program to Eliminate Lymphatic Filariasis (PacELF), American Samoa began a nation-wide mass drug administration (MDA) program in 2000 after baseline surveys indicated 16.5% of 2,989 residents were infected with *Wuchereria bancrofti* based on tests for circulating filarial antigen (CFA). Follow-up surveillance of CFA in four sentinel villages after the implementation of annual MDA with diethylcarbamazine and albendazole showed no significant change in 2001 (11.5%) and 2003 (13.5%). The American Samoa lymphatic filariasis elimination program made improvements to the social mobilization and drug distribution strategies after the 2003 sentinel assessment. The purpose of this project was to measure the impact of MDA after three years of improved community participation resulting from strategic changes in program implementation. The rapid Immunochromatographic Card Test (Binax, Portland, ME) (ICT) was used to test for CFA in a convenience sample of volunteers from the four sentinel villages and one additional village. Positive individuals had a thick blood smear (20 µl) for microfilaria (Mf) testing. Of 1,372 individuals tested (665 men, 707 women), 87.2% reported MDA treatment in 2005. Of persons tested, 13 were ICT positive (0.95%), and two of 13 were Mf positive. Results show a dramatic decline in antigenemia from 13% (2003) to 0.95% (2006). Although results cannot be generalized beyond the villages tested, the decline in CFA prevalence demonstrates the effectiveness of changes made in social mobilization and drug distribution. Additional surveillance is needed to evaluate the progress toward elimination of lymphatic filariasis in American Samoa.

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LONG TERM EFFECT OF MASS DRUG ADMINISTRATION ON THE PRESENCE OF ANTIBODIES AGAINST BM14 AS A MEASURE OF LYMPHATIC FILARIASIS EXPOSURE AND INFECTION IN PAPUA NEW GUINEA

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This study evaluates the change in IgG4 antibodies to the recombinant antigen Bm14 in a population that received four rounds of annual mass drug administration against *Wuchereria bancrofti.* The study population was drawn from a randomized field trial of mass drug administration performed in the East Sepik province of Papua New Guinea from 1993 to 1998. Stored serum samples collected one and five years following the final mass drug administration are compared to pre-treatment values for antibodies to Bm14 as an indicator of transmission exposure and current infection and to circulating antigen using OG4C3 as an indicator of adult worm burden. In a population with a baseline prevalence of 72.4% Og4C3 antigenemia (n=1344), 49.8% remained antigenemic one year following the fourth MDA ($p<0.001$). A subsample of these individuals demonstrated a higher Bm14 antibody prevalence of 88.9% at baseline that decreased to 49.2% one year following the fourth MDA ($p<0.001$). Of particular note is the fact that a large number of children under the age of 6 remained positive for antibody (36.6%) after their villages received four rounds of MDA, indicating ongoing transmission and/or infection among young children in these villages following repeated MDA. Long term follow-up of this population revealed a continued decrease in antigenemia to 14.4% and in antibody prevalence to 31.1% five years following the last MDA. These data suggest that the presence of adult worms as measured by antigen and antibody may remain at low to moderate levels in populations both immediately and over many years following repeated mass drug administration.

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IS THERE CONTINUING TRANSMISSION OF *ONCHOCERCA VOLVULUS* IN THE ESCUINTLA-GUATEMALA FOCUS OF GUATEMALA?

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The goal of the Onchocerciasis Elimination Program of the Americas (OEPA) is to eliminate eye disease due to onchocerciasis in the Americas by 2007 and interrupt transmission where possible. Guatemala has four non-contiguous foci of onchocerciasis transmission and is responsible for 37% of the population at risk for onchocerciasis in the Americas. OEPA has recently recommended to the Ministry of Public Health (MOPH) of Guatemala to suspend biannual Mectizan® treatments of the population of the Santa Rosa focus (9,855 people at risk) after reviewing data from an extensive evaluation that demonstrated no evidence of morbidity or transmission of onchocerciasis. We report here the results of a similar evaluation currently under way in the Escuintla-Guatemala focus (50,588 people at risk). Entomologic evaluations using human landing catches in eight coffee plantations for 1,520 hours of collection from November 2005 through April 2006 resulted in the capture of 23,789 *Simulium ochraceum*, the main vector in the area. Average daily biting rates of *S. ochraceum* were 121 per day. Although this daily biting rate is significantly higher than the estimated minimum required to sustain transmission (i.e. approximately 21 per day), there have been 0 black flies positive for parasite DNA with 60% of the vectors analyzed using PCR to date. We will present the results of a serological survey for antibodies to a recombinant *Onchocerca volvulus* antigen (OV-16) in more than 3000 school children, as well as the results of an ophthalmologic evaluation in more than 300 residents of areas with the highest historical prevalence of onchocerciasis. Results of this evaluation will help guide the Guatemala MOPH as to whether treatment for onchocerciasis can be suspended in this focus and may free more than 50,000 people from the need of bi-annual treatments.

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PERSISTENCE OF *BRUGIA MALAYI* DNA IN VECTOR AND NON-VECTOR MOSQUITOES: IMPLICATIONS FOR XENOMONITORING AND TRANSMISSION MONITORING OF LYMPHATIC FILARIASIS

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Xenomonitoring (detection of parasite DNA in mosquitoes) is a sensitive marker for assessing the endemicity of filarial worms and a useful tool for evaluating elimination programs based on mass drug administration. The purpose of this study was to examine the fate of microfilariae (MF) and filarial DNA in competent and non-competent mosquito vectors. We compared the detection of *Brugia malayi* parasites by dissection and quantitative real-time PCR in three different mosquito strains. These included a competent laboratory vector (*Aedes aegypti* black-eyed Liverpool or *Ae*L), a non-transmitting strain that supports migration

of MF across the midgut to the thoracic musculature with no further development (*Ae. aegypti* Rockefeller or *Ae*R), and a non-transmitting species in which MF do not penetrate the midgut but die within the midgut lumen (*Culex pipiens* or *Cu*). Mosquitoes were studied at 0-4, 7, 10, 14 and 21 days after membrane feeding on infected cat blood. Dissection revealed developing larvae at all time points in *Ae*L but not in the other strains. We used TaqMan and Eclipse MGB real-time PCR assays to detect *B. malayi Hha*I repeat DNA in mosquito DNA samples. Parasite DNA was detected in all *Ae*L and *Ae*R pools by both assays throughout the study period. In contrast, parasite DNA was detected in fewer *Cu* mosquito pools taken at later time points after feeding, and DNA values were lower in *Cu* than in the *Aedes* strains. We conclude that PCR is much more sensitive than dissection for detecting filarial parasites or their remnants in mosquitoes. However, parasite DNA can be detected in both vector and non-vector mosquitoes for two weeks or longer after they ingest infected blood. Thus, although xenomonitoring with vector and non-vector mosquito species may be a sensitive method for indirectly detecting filarial parasites in human populations, positive tests for parasite DNA in mosquitoes do not necessarily prove that transmission is ongoing in the study area.

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INVESTIGATION OF SYSTEMATIC NONCOMPLIANCE IN THE CONTEXT OF A MASS DRUG ADMINISTRATION PROGRAM FOR LYMPHATIC FILARIASIS

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Programs to interrupt transmission of lymphatic filariasis through annual mass drug administration (MDA) are underway in more than 40 countries. Compliance with MDA is a critical determinant of program success because systematically noncompliant individuals may serve as a reservoir for infection. We assessed MDA compliance (n=2378), conducted blood surveys and prepared GPS maps in four selected communities in Leogane, Haiti after four rounds of treatment. We subsequently used a case-control study design to assess differences between noncompliant individuals, defined as persons who never participated in the MDA and compliant individuals, defined as persons who participated 4 years. A survey questionnaire concerning practices, beliefs, and attitudes towards MDA was administered to 367 people. Using a reduced logistic regression model, the odds of being noncompliant were increased for individuals who are female (OR: 3.71). Moreover, fear of side effects (OR: 3.50), lack of knowledge about lymphatic filariasis, and difficulty swallowing treatment pills (OR: 17.76) were shown to significantly increase the odds of being noncompliant in respondents. Some of these concerns can be addressed with new health education messages. We are now working to determine if noncompliance and residual infections are clustered spatially.

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DIAGNOSIS OF LYMPHATIC FILARIASIS INFECTION: HOW MANY PEOPLE HAVE ADULT WORMS?

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Diagnosis of lymphatic filariasis has traditionally been based on detection of microfilariae (mf) in the blood. The sensitivity of mf tests declines at lower mf intensities and mf-negative infections cannot be detected. Only recently it has become possible to demonstrate the presence of adult worms, using standardized antigen detection tests or ultrasound. We did a systematic literature review to obtain insight into the sensitivity of these new tests. Post-treatment data were excluded from our review. Antigen tests have high sensitivity. We found that the community antigenaemia prevalence was on average about twice as high as the mf prevalence, but there was much variation between studies. The relative difference was larger in low-endemic than in high-endemic areas, but it also depended on the type of mf or antigen test. Antigens were detected in nearly all (90%-100%) mf-positive individuals, but the proportion of mf-negatives with antigenaemia varied strongly between studies (~20% on average). Ultrasound has lower sensitivity. Its use is basically restricted to men, in whom the filarial dance sign (FDS, ultrasound registration of the rapid random movements of adult worms) can be detected in the scrotum. About 80% of mf-positive and 20% of mf-negative men were FDS-positive, but these numbers varied between studies. Ultrasound can be applied to superficial lymph nodes in women, but the sensitivity in women was found to be low: FDS was detected in less than half of the mf-positive women. Antigens were not always detected in FDS-positive men: part of the mf-negative (FDS+) men were missed. This suggests that even the highly sensitive antigen test does not detect all infections. A study from East-Africa, that used all three tests in a population survey among males, showed that about 80-90% of all infected individuals (positive on at least one of the three test) were detected by antigen tests. Our results help to estimate the true infection prevalence from measured mf or antigen prevalence levels.

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SAFETY AND EFFICACY OF DOXYCYCLINE THERAPY WITH AND WITHOUT SINGLE DOSE ALBENDAZOLE/IVERMECTIN FOR THE TREATMENT OF MANSONELLA PERSTANS INFECTION

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In Africa, the endemic areas of the filarial parasites *Mansonella perstans* (Mp) and *Wuchereria bancrofti* (Wb) frequently overlap. Unlike Wb, Mp is refractory to standard antifilarial therapies, including diethylcarbamazine, ivermectin and albendazole. The recent discovery of bacterial endosymbionts (Wolbachia) in a number of filarial species, including Mp, has increased the therapeutic options for these infections. We previously reported the early (6 week and 6 month) results of a randomized open-label trial of doxycycline treatment (200 mg daily for 6 weeks) in 225 subjects with Mp infection (80 Mp+ and 145 Mp+Wb+) in Mali. Doxycycline treatment was well-tolerated and led to a modest, but significant reduction in geometric mean Mp microfilarial levels at 6 months following treatment (from 20.6 to 5.8 mf/60µl blood). Subjects coinfected with Mp and Wb were subsequently randomized to receive a single dose of albendazole 400mg plus ivermectin 200µg/kg or no further treatment. Microfilarial levels were measured 6 months later (1 year after the administration of doxycycline). Mp microfilaremia cleared completely at 12 months in all 18 subjects who received doxycycline alone and in 41 of 43 subjects who received doxycycline followed by albendazole/ivermectin ($p<0.05$ as compared to the no treatment group). Although there was a significant decrease in Mp %pre-treatement levels in the group who received only albendazole/ivermectin (70% vs. 41%, $p = 0.005$), microfilarial clearance was observed in only 3 of 18 (7%) of the subjects who received albendazole/ivermectin alone and in 5 of 43 (11%) subjects who received no treatment (p =NS). As expected, Wb microfilarial levels and prevalence fell significantly in response to all three drug regimens. In conclusion, doxycycline is safe and effective for the treatment of Mp infection. The effect of doxycycline is not enhanced by a single dose of albendazole/ivermectin, consistent with the previously described poor efficacy of these drugs against Mp.

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FUNCTIONAL POLYMORPHISM IN THE IL-1 B -31 GENE PROMOTER (C-T) IS ASSOCIATED WITH PROTECTION AGAINST SEVERE MALARIAL ANEMIA IN INFANTS AND YOUNG CHILDREN

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Interleukin (IL)-1 β is a pro-inflammatory cytokine released as part of the innate immune response to *Plasmodium falciparum*. Although the role of IL-1 β in the pathogenesis of severe malarial anemia (SMA) remains undefined, polymorphic variability in IL-1 β conditions disease outcomes in infectious and autoimmune diseases. To examine the role of genetic variation in IL-1 β on conditioning severe malaria disease outcomes in children in a holoendemic *P. falciparum* transmission area, the relationship between IL-1 β -31C/T polymorphism and high-density parasitemia (HDP; 10,000 parasites/μL or greater) and SMA (Hb<6.0 g/dL) was investigated. Functionality of IL-1 β -31C/T was examined by determining the association between circulating IL-1 β levels and malaria disease outcomes. Children with acute malaria (n=569) were enrolled at Siaya District Hospital in western Kenya. Complete blood counts were determined using a Beckman-Coulter CounterTM and Giemsa-stained slides were used to determine parasite density. IL-1 β -31 genotyping was carried out by PCR and Alu I restriction enzyme digestion. IL-1 β plasma levels were determined with the Human Cytokine Multiplex Antibody Bead Array. Prevalence of CC, CT, and TT genotypes were; 65.2%, 20.9%, and 13.9%, respectively, with an allelic distribution of p=0.75 and q=0.25 for the C and T alleles, respectively. Multivariate logistic regression revealed that homozygous T alleles protected against SMA (Hb<6.0 g/dL; OR; 0.48, 95% CI, 0.28-0.84; P=0.02). Relative to the CC genotype, heterozygotes had increased susceptibility to HDP (OR; 2.55, 95% CI, 1.47-4.42; P=0.001), while the TT genotype had a mildly elevated risk of HDP (OR; 1.39, 95% CI, 0.79-2.46; P=0.25). Furthermore, the TT genotype was associated with significantly higher IL-1 β levels compared to the other genotypes (P<0.05 for both groups). Results presented here demonstrate that homozygosity of the -31 T allele is associated with protection against SMA and functionally higher IL-1 β production during acute malaria.

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INITIATION OF ANTI-SPOROZOITE IMMUNITY IN MALARIA IS EXTRA-HEPATIC, WHILE THE EFFECTOR PHASE OPERATES WITHIN THE LIVER

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Immunization with gamma-irradiated sporozoites of the malarial parasite *Plasmodium*, confers immunity against subsequent challenge with live parasites. Dendritic cells that capture antigen from infected hepatocytes, are thought to prime naive T cells in the liver or hepatic lymph nodes to initiate the cellular immune response. Alternately, natural infections via mosquito bites may in fact allow sporozoite antigens to reach the skin draining lymph nodes (DLN) and prime T cells here. We tested the latter possibility by evaluating the CD8 positive T cell response to the circumsporozoite protein (CS) in various tissues, after P. yoelii immunization by infected mosquito bites or direct sporozoite-injection in the ear. Using single cell IFN-γ secretion assays, a CS specific CTL response was evident in the draining auricular LN just two days after immunization. Specific activated CTLs were absent from the celiac LN, contralateral auricular LN, liver or spleen, where they appeared only by four days. When the draining auricular LN was surgically ablated to prevent the early local priming, total numbers of IFN-γ-secreting CTLs in the liver were reduced by 54%, indicating that the DLN contributes significantly to the generation of effector CD8 cells. Protective immunity to intravenous sporozoite challenge was also drastically curtailed, if the DLN and spleen were ablated before immunizing through the ear. Although priming of CD8 positive T cells apparently occurs outside the liver, recent data suggests that the effector phase of CTL activity upon subsequent sporozoite challenge takes place within the liver. Our study highlights for the first time, the critical importance of skin-draining lymph nodes in initiating an immune response to Plasmodium sporozoites entering through their natural route. Our data also provides compelling evidence that the inductor and effector sites of anti-sporozoite immunity in malaria are distinctly different cellular compartments, and comprise of lymphoid and hepatic tissues respectively.

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HUMAN SYNCYTIOTROPHOBLAST CELLS PLAY AN ACTIVE ROLE IN THE IMMUNE RESPONSE TO PLACENTAL MALARIA

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Pregnant women, especially primigravidae, are more susceptible to malaria even in endemic areas where substantial acquired immunity in adults is apparent. This often results in what is referred to as placental malaria (PM): the sequestration of malaria-infected red blood cells (iRBCs) and infiltration of maternal leukocytes in the placental intervillous spaces (IVS). PM is a major public health problem associated with poor fetal outcomes such low birth weight. While the accumulation of iRBCs is known to be mediated at least in part by the binding of iRBCs to syncytiotrophoblast (ST), fetal cells in direct contact with maternal blood, little is known about how this accumulation influences ST function. We recently described an *in vitro* system using primary human trophoblast that is useful for the assessment of biochemical and immunological changes induced in ST by specific binding of ST-adherent iRBCs (iRBCST). In this study, we further characterized the immunologic impact of malarial parasite/ST interactions. The binding of iRBCST to ST induced increased activation of the mitogen activated protein kinase (MAPK) JNK and the chemotaxis of normal peripheral blood mononuclear cells. Studies are currently under way to identify the chemotactic factor(s) involved in this phenomenon. Stimulation of ST with crude malarial antigens and hemozoin led to increased phosphorylation of ERK1/2 MAPK. Treatment with hemozoin stimulated the secretion of IL-8, but neither this treatment nor iRBCST binding induced ST to secrete MIP-1a or -1b which have been reported in the IVS of the malaria-infected placenta. Taken together, these results suggest that the ST influences the local immunological milieu during PM. Importantly, the ST could contribute to recruitment and retention of maternal leukocytes in the IVS. Determining the relative roles of maternal leukocytes, ST and other fetal cell types in this process is essential for the understanding of the induction of both protective and pathogenic immune responses to malaria in the placenta.

(ACMCIP Abstract)

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PLACENTAL MALARIA DECREASED PLASMOCYTOID DENDRITIC CELLS IN BLOOD FROM PREGNANT WOMEN IN WEST AFRICAN AREA

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During pregnancy immuno-supression occurs permitting fetus implantation and growth. This suppression is related to a TH2 switch of the immune response, mostly triggered by dendritic cells (DCs). During placental malaria, parasites accumulate in the intervillous spaces of the placenta and can modulate DCs activity. DCs have three subsets: plasmocytoid (pDC), myeloid (mDC) and less differentiated (ldDC). The aim of this study was to compare DCs subsets, maturation and cytokine production, in peripheral, placental and cord blood between healthy and parasitized primi/secondigravid. The blood samples were obtained at delivery after informed consent. DCs were analyzed by flow cytometry and identified in total blood as Lin1-negative and HLA-DR positive cells. CD11c and CD123 were used to identify mDC and pDC respectively. Maturation of the DCs was evaluated using HLA-DR and CD83 expressions. Cytokines production was measured in peripheral and cord blood, using CBA human Th1/Th2 kit (BD).

In infected pregnant women we observed a significant decrease of pDCs according to uninfected pregnant women, both in peripheral blood (8±3% versus 20±3%), placental blood (17±3 % versus 24±2 %) and cord blood (10±2% versus 20±2%). HLA-DR expression also showed a severe decrease for the three subsets of DCs both i) in venous blood: mDCs (662±98 versus 1301±2), pDCs (415±40 versus 672±69 UA), ldDCs (388±61 versus 585±47); ii) in placental blood: mDCs (662± 110 versus 1421± 108 UA), pDCs (270,± 39 versus 438± 41 UA), ldDCs (191±30 versus 519±52 UA); iii) and in cord blood : mDCs (887±118 versus 1182±83 UA), pDCs (306±47 versus 412±39 UA) and ldDCs (295±38 versus 406±52 UA). However, we found a significant increase of IFN-γ in peripheral blood (273±56 versus 103±16 pg/mL) and in cord blood (201 ± 33 pg/mL versus 88 ± 16 pg/mL) during infection. IL-10 also increased in peripheral blood (101±20 versus 16±4 pg/mL) and in cord blood (34±5 versus16±4 pg/mL).

These findings suggest a modification of DCs profile, maturation and cytokine production during placental malaria infection.

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MALARIA-EXPOSED MEN AND PREGNANT WOMEN DEVELOP DIVERGENT ANTIBODY RESPONSES TO VAR1CSA AND VAR2CSA PROTEIN DOMAINS

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*Plasmodium falciparum-infected erythrocytes (IE) adhere to cho*ndroitin sulfate A (CSA) to sequester in the human placenta, and placental malaria is associated with disease and death of both mother and child. Susceptibility to pregnancy malaria decreases over successive pregnancies as women acquire antibodies against CSA-binding IE. Proteins encoded by var1csa and var2csa have been implicated in binding to CSA, and both have been proposed as vaccine candidates for pregnancy malaria, although the evidence relating var1csa to placental malaria has been questioned. To study the involvement of these molecules in protective immunity, we prepared arrays of their individual domains and tested their reactivity with sera from East African females of varying gravidity, East African males, and non-immune individuals from non-endemic areas. To improve chances to re-create structural epitopes, we designed a novel vector that expresses malaria antigens on the surface of mammalian cells as GFP fusion proteins. After expression in COS-7 cells, antigens were purified and immobilized in 96-well plates in a single-step procedure, and serum reactivity was normalized to the abundance of antigen measured in individual wells. The reactivity to several var2csa domains was higher with sera from multigravid females than males, and was also parity related (multigravid > primigravid). Conversely, reactivity to var1csa domains was consistently higher with sera from males, and this relationship was significant for several var1csa domains. The results suggest that humoral immunity decreases to one parasite variant as it increases against another, and are consistent with a role for var2csa but not var1csa in placental malaria.

(ACMCIP Abstract)

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LIMITED GLOBAL DIVERSITY OF ANTIBODY EPITOPES EXPRESSED BY PLACENTAL BINDING *PLASMODIUM FALCIPARUM* VARIANTS

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Pregnant women are particularly susceptible to *Plasmodium falciparum* malaria, which is a major cause of infant and maternal morbidity and mortality. Variants of *P. falciparum*-infected erythrocytes (IEs) that cause malaria in pregnant women exploit gaps in the repertoire of variant specific immunity and adhere in the placental vascular spaces. Antibodies to surface antigens expressed by these variants are associated with protective immunity. The major target antigen is PfEMP1, which is a highly polymorphic protein encoded by the var multigene family. PfEMP1 variants encoded by var2csa-type genes mediate adhesion of IEs to chondroitin sulfate A and other placental receptors. Defining the global diversity and distribution of epitopes expressed by placental-binding variants is essential for understanding acquired immunity and vaccine development, as well as the evolution of polymorphisms and possible constraints on diversity. To investigate these questions, we have examined antibody recognition of several defined placental-binding isolates that express polymorphic variants of var2csa among widely geographically-separated populations in Kenya, Malawi and Papua New Guinea. All placental-binding variants were commonly recognized by exposed pregnant women in a gender and parity-dependent manner and antibody levels were higher among women with placental malaria than uninfected pregnant women. Antibody epitopes are not strictly conserved as many individuals demonstrated isolate-specific antibodies rather than pan-reactive or cross-reactive responses; however cross-reactive antibodies were observed in some women. Recognition of the same polymorphic variants suggests that there are common epitopes expressed by placental-binding variants that are globally restricted and have a wide geographic distribution. Immunity may be mediated by a repertoire of antibodies to common variants raising the possibility that such responses could be induced by a multivalent vaccine that incorporates several common epitopes.

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CHARACTERISATION OF THE ANTIBODY RESPONSE AGAINST *PLASMODIUM FALCIPARUM* ERYTHROCYTE MEMBRANE PROTEIN-1 IN HUMAN VOLUNTEERS

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The immune response against the *Plasmodium falciparum* variant surface antigen, PfEMP1 is a key component to the development of clinical immunity. The characteristics of the anti-PfEMP1 response including the trigger, the specificity and longevity of the response remain to be elucidated. In this study we used the serum from human volunteers who were infected with a known inoculum of 3D7 to investigate the development, specificity and dynamics of anti-PfEMP1 antibodies to a known PfEMP1 DBL1⟨ repertoire represented by 6 different DBL1⟨ fusion proteins. We have shown that a parasitemia between 5 and 200 infected red blood cells per µl is required for initiating an antibody response to DBL1⟨ and that re-exposure to a smaller number of parasites significantly boosts the response. We demonstrated that the antibody response consists of both DBL1⟨ variant-specific and cross-reactive IgGs. The variant-specific IgGs were able to agglutinate IRBC, while inhibition of rosette formation appears to be attributed to cross-reactive IgGs.

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MITOCHONDRIAL DIVERSITY, GENE FLOW, AND PHYLOGEOGRAPHY IN THE TSETSE FLY GLOSSINA PALLIDIPES

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A widely but discontinuously distributed savanna species, ***Glossina pallidipes*** is one of the economically most important tsetse flies. Its bionomics seem to differ regionally and genetic studies are indicated.

DNA sequences at ribosomal (r16S, 249 bp) and cytochrome oxidase (COI, 421 bp) mitochondrial genes were analyzed in thirty-three geographically disparate samples of G. pallidipes from two southern (Zambia, Zimbabwe) and three eastern African (Tanzania, Kenya, Ethiopia) countries. One hundred and twenty-two composite haplotypes were detected and their spatial frequency distributions were heterogeneous. Sixteen (13%) haplotypes were private, 79 (65%) were singular and only two haplotypes were shared between southern and eastern African populations. The haplotype frequency distribution conformed to the expectations of the infinite allele mutation model. Spatio-temporal correspondence analysis clustered populations into three groups, southern Africa, East Africa, and Ethiopia.

Haplotype and nucleotide diversities were much greater in eastern Africa than southern Africa. Little haplotype and nucleotide diversity was shared among countries, indicating limited gene flow. Genetic differentiation was three times greater among than within countries, indicating a high degree of population structuring. Temporal variation in haplotype frequencies afforded effective population size estimates for three locations. Discussed briefly is the relevance of geographical mitochondrial heterogeneity to gene flow, phylogeography, and vector competence.

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LOSS OF GENETIC DIVERSITY FOR *FRANCISELLA TULARENSIS* INFECTING DOG TICKS WITH INCREASING EPIZOOTIC DURATION

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During the last 6 years the island of Martha's Vineyard, Massachusetts has experienced an epizootic of tularemia due to *Francisella tularensis* (Ft). Although the mode of perpetuation of the agent of tularemia there remains undescribed, our work suggests that dog ticks, *Dermacentor variabilis*, play an important role. Multi-locus variable number tandem repeat analysis (MLVA) demonstrates that Ft in dog ticks on the island comprises diverse haplotypes; using 2 informative loci, we calculated a Simpson's index of 0.86. This amount of diversity is consistent with the hypothesis that Ft has been enzootic on MV since its introduction in the 1930s. It may be that between epizootics Ft is maintained in small isolated natural foci. Epizootics occur when local amplification causes natural foci to coalesce. In such a scenario, the greatest diversity would be evident during the initial stages of the epizootic as previously isolated foci of transmission merge together. To test this hypothesis, we examined the diversity of Ft in dog ticks from a single site on MV over 4 years using MLVA. Questing dog ticks were collected by flagging from April to August 2002-2005 and tested for evidence of Ft infection using a nested PCR for the fopA gene. Positive samples were reamplified using 2 MLVA loci. Simpson's index of diversity was thereby calculated for each summer of sampling. Over the course of the study, the diversity index fell linearly from 0.87 in 2002 to 0.36 in 2005. During this time the prevalence of infection in host-seeking ticks rose from 0.6% in 2002 to greater than 2.5% in all subsequent years. The decrease in diversity, despite the increase in prevalence, appears to be due to the emergence of a few dominant haplotypes. These findings suggest that certain haplotypes are more readily transmitted, and are consistent with the hypothesis of epizootic development as a result of the expansion of natural foci.

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A NEW PROTOCOL FOR THE DETECTION AND IDENTIFICATION OF RICKETTSIAE IN TICKS REMOVED FROM MILITARY PERSONNEL

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Tick transmitted rickettsiosis is a risk to military personnel stationed in the United States and overseas. Tick-borne rickettsiae, including *Rickettsia rickettsii*, the causative agent of Rocky Mountain spotted fever, have been associated with febrile disease outbreaks among soldiers in Arkansas, Virginia and Botswana. A polymerase chain reaction and restriction fragment length polymorphism (PCR/RFLP) protocol identified *Rickettsia montanensis*, but not *R. rickettsii* in 308 *Dermacentor variabilis* ticks submitted to the Human Tick Test Kit Program of the U.S. Army Center for Health Promotion and Preventive Medicine in 1997. For this study we have developed and used a more rapid and specific method to detect and identify rickettsiae from *D. variabilis* and *Amblyomma americanum* ticks submitted to the Human Tick Test Kit Program in 2001 - 2003. This protocol utilizes a quantitative real-time PCR (Rick17 qPCR) that detects the *Rickettsia* genus specific 17 kDa common antigen gene to screen ticks for the presence of rickettsiae. Subsequently, qPCR assays targeting a species-specific portion of the outer membrane protein (OmpB) gene are used to detect three tick-borne rickettsiae: *R. rickettsii, R. montanensis,* and *R. amblyommii*. Agents detected by Rick17 qPCR, but not identified

in the three species-specific qPCR assays, are then evaluated using multilocus sequence typing (MLST). Of approximately 1,000 *D. variabilis*, 237 ticks have been assessed, 11 were positive for *R. montanensis*, 221 were negative and 5 are being evaluated by MLST. Of approximately 800 *A. americanum* ticks, 44 tick pools were assessed and 36 were positive by Rick17 qPCR, and of those 22 were positive for *R. amblyommii*. The fourteen Rick17 qPCR positive samples that were not positive by the species-specific qPCR are being evaluated by MLST. This system for detecting and identifying tick-borne rickettsiae provides a new mechanism for assisting in the diagnosis of rickettsioses and in the assessment of the risk of tick-borne rickettsial diseases to military personnel.

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DURATION OF TICK ATTACHMENT NECESSARY FOR TRANSMISSION OF *ANAPLASMA PHAGOCYTOPHILUM* TO A SUSCEPTIBLE VERTEBRATE HOST

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The duration of attachment by an infected tick that is necessary for successful transmission of a pathogen is an important factor which determines how soon an attached tick must be removed in order to prevent an infection. The agent of human granulocytic anaplasmosis has been found in hemocytes of unfed ticks *I. scapularis,* which indicates the generalized infection and thus a potential for "immediate" transmission following the attachment of an infected tick. However, several reports suggest the delayed transmission of *A. phagocytophilum*. We determined the duration of tick attachment necessary for transmission of *A. phagocytophilum* from nymphal *I. scapularis* to susceptible vertebrate hosts. Individual nymphs were placed upon BALB/c mice and allowed to attach for various intervals between 4 and 78 hours. At predetermined intervals, ticks were remover and tested for the presence of *A. phagocytophilum* DNA. The success of transmission was determined by the presence of the agent DNA in mouse blood tested at 7, 14 and 21 days postinfestation, and specific IgM/IgG antibodies in sera tested by IFA at 21 days postinfestation. 17 to 33% of infected ticks attached for up to 16 hours were able to transmit *A. phagocytophilum* to susceptible animals. This proportion reached 60% in those allowed to feed for 24 hours. The transmission success reached 93% in ticks attached for 78 hours. Although, only a small proportion of infected nymphal *I. scapularis* were capable of transmitting *A. phagocytophilum* immediately after the attachment, the risk of infection in susceptible animals almost tripled within the first 24 hours. Interestingly, none of the PCR-positive mice that were exposed to infected ticks for less than 12 hours and only 50% of those PCR -positive mice exposed for 12 to 20 hours seroconverted within 3 weeks postinfestation. Mice that turned PCR-positive after being infested for at least 24 hours all seroconverted. This suggests that although ticks feeding for less than 24 hours do inject small amount of *A. phagocytophilum*, this amount is insufficient for establishing a potent generalized infection in BALB/c mice. It seems likely that although *A. phagocytophilum* is present in salivary glands of unfed *I. scapularis* nymphs, the amount of *A. phagocytophilum* contained in saliva is insufficient for causing an infection in a host, and replication of the agent for up to 20-24 hours is required in a feeding tick before a successful transmission can occur.

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DEMOGRAPHIC HISTORY AND POPULATION STRUCTURE OF AN EMERGING DISEASE VECTOR, *AMBLYOMMA AMERICANUM*, AND ITS POTENTIAL COEVOLUTION WITH "*RICKETTSIA AMBLYOMMII*"

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Amblyomma americanum is a particularly aggressive tick species that feeds on humans in all life stages. Public health interest in *A. americanum* has grown recently due to the discovery that it transmits several newly recognized human pathogens and the fact that the distribution of the tick is expanding. We investigated the population structure of this tick and "*Rickettsia amblyommii*" to test for coevolution and the null hypothesis of isolation by distance. A hierarchical population genetic study was conducted on over 2000 individual *A. americanum* ticks from over 30 populations throughout the range of *A. americanum*. All but one population of *A. americanum* were infected with "*R. amblyommii*" and the genetic diversity of this agent is being examined. Denaturing high performance liquid chromatography (DHPLC) with the WAVE® Nucleic Acid Fragment Analysis System was used to detect single nucleotide polymorphisms and indels in a 316 bp fragment of the mitochondrial 16S gene and a 389 bp fragment of the ribosomal internal transcribed spacer 2 (ITS2). Each unique sequence produced distinctive chromatogram peaks resulting in detection of thirty-one haplotypes for 16S and twenty haplotypes for ITS2. A minimum spanning tree was constructed and indicated that these haplotypes comprised two lineages. The majority of the variation found was among ticks within each population, indicating high amounts of gene flow between geographic regions. However a significant overall FST value indicates genetic differentiation between collection sites, despite Mantel regression analysis revealing no isolation by distance. Mismatch distribution analysis and tests of neutrality indicated expansion in several populations, and admixture events or population subdivision followed by expansion in others. The high degree of gene flow found within *A. americanum* may be due to bird mediated dispersal of ticks which may have significant impact on the prevalence of pathogens within this important tick vector.

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RICKETTSIAL DISEASES IN NORTH CAROLINA: IS "RICKETTSIA AMBLYOMMII" A POSSIBLE CAUSE OF RICKETTSIOSIS REPORTED AS ROCKY MOUNTAIN SPOTTED FEVER?

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Rocky Mountain spotted fever (RMSF) is the most frequently reported tick-transmitted illness in North Carolina. In 2005, approximately 1,843 cases of RMSF were reported to CDC nationally with 625 (33.9%) cases reported from NC. We initiated an enhanced surveillance project for tick-transmitted illnesses in Chatham County, NC from May - December 2005. Ticks (n = 6,502) were collected by flagging woodland vegetation around 32 home sites from May - July. *Amblyomma americanum* (99.6%) was most abundant with few *Dermacentor variabilis* (0.4%), and *Ixodes scapularis* (< 0.1%) collected. Of tick pools tested (n = 51) by PCR assays, Rickettsia spp. were detected in 21 pools. Sequence analysis of 17 pools identified "*Rickettsia amblyommii*" in 11 *A. americanum* pools and in 2 single-*D. variablis* extracts. Specific identifications could not be made for 4 pools of *A. americanum*. Differentiation between *R. rickettsii* and *R. montana* could not be made in 2 D. variablis pools and in the single I. scapularis tested. In another single *D. variablis* female, we could not differentiate between *R. felis* and *R. rickettsii*. Single (n = 22) and paired sera (n = 29) from suspected human cases were tested for antibodies reactive with *R. rickettsii*, using an IFA. Sixteen patients were classified as probable RMSF cases. Paired sera for 6 probable RMSF patients were further tested by IFA against a *R. rickettsii* antigen as well as "*R. amblyommii*" antigen because of the high prevalence of this agent. IFA titers were significantly greater for "*R. amblyommii*", suggesting exposure to "*R. amblyommii*" or another unevaluated spotted fever group rickettsia relative to *R. rickettsii*. Dried blood collected on Nobuto paper strips

from 32 white-tailed deer (*Odocoileus virginianus*) and eluted in PBS were tested by IFA for antibodies to "*R. amblyommii*" and *R. rickettsii*. Results of IFA tests on deer sera are pending. "*Rickettsia amblyommii*" is a possible cause of the escalating number of RMSF cases reported in recent years, and deserves further attention as a potential emerging human pathogen in NC.

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THE 47 KDA ANTIGEN OF *ORIENTIA TSUTSUGAMUSHI* KARP STRAIN PROVIDED HETEROLOGOUS PROTECTION IN A MOUSE LETHAL CHALLENGE MODEL

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Scrub typhus is an acute, febrile disease caused by infection with *Orientia tsutsugamushi*. At the present time there is no vaccine for scrub typhus. Strain variation is the major concern in developing scrub typhus vaccine since *Orientia* exhibits extensive antigenic variations and previous vaccine efforts have demonstrated that cross-protection against heterologous strains is very poor. In order to develop a broadly protective vaccine, we have constructed a DNA vaccine plasmid expressing the 47 kDa antigen of Karp strain (pKarp47). Unlike the variable major outer membrane protein 56 kDa antigen, this 47 kDa antigen is relatively conserved among different strains. To evaluate the protective efficacy of pKarp47, Swiss CD-1 out bred female mice were immunized intramuscularly once with 100 µg of pKarp47 and 10 µg of a plasmid expressing GM-CSF (pGM-CSF) as an adjuvant. Four weeks after immunization, mice were challenged with homologous and heterologous strains of *Orientia* and the morbidity and mortality were monitored daily. We consistently observed 70-100% homologous protection against Karp strain. Among the 17 heterologous strains evaluated, pKarp47 provided 80-90 % protection against strains TH1812, TH1814 (both are from Thailand) and strain18032460 (Malaysia) and 40% protection against Citrano (Australia) and TH1811 (Thailand). Although no protection against other strains can be demonstrated, delay of death in immunized mice were observed almost in every cases. Considering the rapid induction of protective immunity after single immunization and the partial cross protection against heterologous strains by pKarp47, a mixture of few strains of 47 kDa DNA vaccine has the potential to provide broad protection for various strains of *O. tsutsugamushi*.

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DENGUE SURVEILLANCE IN ARAGUA STATE, VENEZUELA: A TEN-YEAR PERIOD RETROSPECTIVE ANALYSIS

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Outbreaks of Dengue Fever (DF) had occurred in Venezuela since the 1950s, but Dengue hemorrhagic fever (DHF) first emerged in Maracay, Aragua State, in October 1989 and extended rapidly all around the country producing a prolonged outbreak until April 1990. Afterward, DF and DHF have become endemic with annual outbreaks occurring mostly during the rainy season. In 1996, a surveillance system was created in Aragua which included all public clinics and hospitals as sentinel centers and a reference diagnostic laboratory. Throughout 10 years, the laboratory-based surveillance system tested sera from 27, 796 suspected patients using RT-PCR, virus isolation in C6/36 cells and IgM-capture ELISA techniques. In this report, based on a ten-year retrospective analysis of 14,868 laboratory confirmed cases, we describe the annual dengue transmission in terms of: attack rates (x100,000 inhabitants), disease presentation, age groups affected, male-to-female ratios, geographic origin, transmission periods, and spatial and temporal circulation of DENV serotypes. Summarizing: a) attack rates ranged from 16.8 to 340.9; b) DF to DHF ratios ranged from 2:1 to 32:1; c) children and adolescent aged 20 days to 14 years were the most affected (range: 61.8% - 72.6% confirmed cases); d) male-to-female ratios ranged from 0.9:1 to 1.1:1, e) dengue cases were confirmed in all 17 state municipalities with variable degree of prevalence rates; f) two periods of transmissibility were defined: a low transmission period from January to May, and a high transmission period from June to December; annual outbreaks generally occurred during the rainy season (May to October); and g) DENV-1, 2 and 4 were the only prevalent serotypes from 1997 to September 2000, predominantly in seven central-northern municipalities, then DENV-3 was re-introduced and all four serotypes circulated afterwards.

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PEDIATRIC COHORT STUDY OF DENGUE TRANSMISSION IN NICARAGUA

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A prospective cohort study was established in Managua, Nicaragua, to better understand the natural history of dengue transmission, to provide a platform for collection of biological samples linked with clinical and epidemiological information, and to establish infrastructure for potential Phase III trials of candidate tetravalent dengue vaccines. The cohort of ~3,500 children 2-9 years of age was recruited in 2004 from the catchment area of the Health Center Socrates Flores Vivas (HCSFV); annual samples are taken at the beginning of the dengue season (July-August) of each year, and detection of dengue occurs passively through attendance at the HCSFV. In Years 1 and 2 (Y1 and Y2), an average of 3% of the 16,526 initial consults (representing 89% of the cohort) were suspected dengue, 2.5% were fever of unknown origin (FUO), 42% were fever with a non-dengue etiology, and 52.5% were for health concerns without fever. All cases of suspected dengue and FUO were evaluated for DEN infection, and 17 and 62 were confirmed as positive in Y1 and Y2 respectively. Case detection was 10-to 20-fold higher at the HCSFV than at all other health centers in Managua in both years. Y1 was characterized by younger age of symptomatic dengue (mean 5.5, mode 7), greater DENV1 circulation (64% DENV1, 21% DENV2, 7% DENV4, and 7% DENV1&4), more primary infections (65% primary, 35% secondary) and a high suspected dengue to FUO ratio among confirmed positives. During Y2, the age of disease increased (mean 7.2, mode 9), more DENV2 circulated (42% DENV1, 56% DENV2, 2% DENV1&2), more secondary infections were observed (43% primary, 57% secondary), and there was an increase in undifferentiated fever among positive cases. Seroprevalence of anti-DENV antibodies ranged from 26% to 91% in 2- to 9-year olds, and the incidence of primary and secondary DENV infections during Y1 was 4.0% and 6.1% respectively. Ninety-six percent of febrile cases attend the health center within 72 hours of fever onset, and home visit surveys indicate that only 0.3-1.1% of the cohort attend other health services when ill with fever. New technologies, including PDAs, barcodes, fingerprint scanning, GIS, and database automatization, were incorporated to streamline procedures and greatly improve quality control. Thus, a pediatric cohort study has been successfully implemented in Managua, with high study compliance.

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IMPACT OF EVIDENCE-BASED COMMUNITY-DERIVED INTERVENTIONS FOR THE CONTROL OF THE DENGUE VIRUS VECTOR *AEDES AEGYPTI* IN MANAGUA, NICARAGUA

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Dengue prevention continues to rely on controlling the *Aedes* mosquito vectors. An ongoing challenge in community-based vector control is to bridge the gap between community knowledge and sustainable practice that reduces mosquito breeding sites. Here we present results from the first 2 years of a project using evidence-based community-derived interventions for the control of *Ae. aegypti* in Managua, Nicaragua. A stratified cluster sample of 30 sentinel sites (130 houses each, for a total of ~3,960 households and ~23,300 individuals) were selected to represent the population of Managua. Seven intervention barrios were initially chosen in 2004 (Y1), and 3 more were added in Y2, with the rest serving as reference barrios. The community-derived interventions are based on 1) "beyond-KAP" questionnaires, 2) entomological surveys, and 3) dengue virus (DENV) infection in children ages 3-9, determined by IgG ELISA of paired saliva samples collected in October and February of each year. With respect to behavioral indicators, a significant improvement in preventative actions to interrupt the mosquito life cycle was found in the intervention barrios. In Y2, intervention barrio residents were significantly more likely to search for and to eliminate larvae/pupae every week on their own than were residents of reference barrios. They were less dependent on insecticides, they spoke more with their neighbors about dengue prevention, and they were more likely to identify community leadership. Entomological indices decreased in intervention compared with reference barrios by Y2, with an OR for homes positive for *Ae. aegypti* pupae of 0.7 (95% CI 0.6-0.9) in Y2 vs 1.3 (1.0-1.6) in Y1 and an OR for miscellaneous containers positive for larvae or pupae of 0.8 (0.7-0.9) in Y2 vs 1.3 (1.1-1.6) in Y1. In addition, in Y2 the average number of pupae in water storage barrels in intervention barrios was 42% of that found in reference barrios. An analogous decrease in the number of children infected with DENV was observed, from equivalent numbers in intervention vs reference barrios in 2004 with an OR of 0.94 (0.74-1.18) to an OR of 0.71 (0.48-1.05), p=0.07 in 2005. Overall, these represent promising advances towards community-based sustainable control of the dengue vector *Ae. aegypti*.

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VIRUS EVOLUTION DURING A SEVERE DENGUE EPIDEMIC IN CUBA, 1997

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Considering the unique epidemiological setting of dengue in Cuba, key observations have been made: i) the association of DHF/DSS with secondary infection and ii) increased clinical severity through time during the epidemics. Accordingly, a hypothesis has been proposed which suggests that the increased severity might be explained by the appearance of neutralization resistant escape mutants. We investigated these ideas by sequencing Dengue 2 virus isolates obtained at different times during the outbreak occurred in Santiago de Cuba, 1997. Initially, 20 Cuban Dengue 2 isolates were studied at the envelope gene. Total conservation of the E gene sequence for these isolates suggests that the selection of envelope gene escape mutants was not the determinant of increased disease severity at least in this epidemic. Additionally, the Maximum Likelihood (ML) phylogenetic analysis indicated that our isolates group with the "American/Asian" genotype. It is noteworthy, a particular amino acidic change observed for the Cuban-Venezuelan-Martinique isolates that is located in an important antigenic region that contains multiple T- and B-cell epitopes and may play a role in its virulent potential. Subsequently, full-length genomic sequences from 6 isolates were determined. Genome analysis revealed strong conservation of the structural proteins and the non-coding regions. Nucleotide substitutions were observed in non-structural genes and most notably in the NS5 gene. Although whether or not the observed changes were fixed by natural selection or genetic drift was uncertain; the ML phylogenetic analysis revealed a clear division within the Cuban strains, with the isolates sampled later in the epidemic forming a separate phylogenetic group, indicating that viral evolution had occurred during the epidemic period. The earliest isolates sampled differed from those sampled later by amino acid replacements in the NS1 and NS5 proteins. Further studies are therefore required to define the functional role of amino acid replacements observed and their possible relation to disease severity.

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DENGUE VIRAL SEQUENCE ANALYSIS FROM BOTH HUMAN AND MOSQUITO SAMPLES ISOLATED DURING CLUSTER INVESTIGATIONS IN KAMPHAENG PHET, THAILAND

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We performed molecular analyses on viruses collected during cluster investigations initiated around a dengue RT-PCR positive index case. Active surveillance was conducted from June to November. Index cases that identified from school absences in our prospective study of dengue virus transmission in primary school children in Kamphaeng Phet, Thailand. We have isolated 17 viruses, 8 from mosquitoes and 9 from humans. Of these, 10 DENV-4 isolates were directly sequenced from 4 mosquitoes and 6 humans, four DENV-3 isolates, two from mosquito and two from human cases, and two DENV-2 isolates, one from mosquito and one from human. We determined the diversity between the mosquito and human samples as well as the sequence variations within and outside the local of the clustered samples. Two DENV-4 clusters investigations accounted for 8 of the E region sequences analyses allowing us to examine and define changes within the E regions that differ in the short 3 month period between the two clusters. We show that there is complete homogeneity within a cluster between mosquitoes and human isolates but distinct differences both at the nucleic and amino acid level that occur during the 3 months between the DENV-4 clusters. Furthermore, E sequencing from DENV-2 and DENV-3 clusters show similar trends with identical human and mosquito viruses. These later samples will be further analyzed against circulating viruses outside the cluster to determine if this homogeneity falls outside these distinct clusters.

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COMPARATIVE ANALYSIS REVEALS GENETIC DIFFERENCES OF DENGUE VIRUSES ISOLATED FROM PATIENTS DURING THE PERIODS OF HIGH, INTERMEDIATE AND LOW TRANSMISSION

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Potential genetic determinants of dengue virulence and genetic differences among dengue viruses (DENV) associated with regional transmission rates (high, intermediate and low) were studied by performing a comparative analysis of gene sequences in the coding region of thirty-two DENV strains isolated between 1973 and 2001, from patients with varied disease severity admitted to the Queen Sirikit National Institute of Child Health (QSNICH), Bangkok, Thailand. A comparative analysis revealed: (1) Genetic variation among the structural and non-structural genes of DENV strains increased concurrently with increases in DENV transmission. More genetic variation occurred in DENV strains sampled during the high transmission period. (2) Two specific amino acids of DENV-3 were associated with the transmission rates. Serine at position 27 of the capsid gene, and lgycine at position 15 of the preM/M gene correlated with high transmission, while threonine at position 27 of the capsid gene and alanine at position 15 of the preM/M gene correlated with low transmission. (3) No consistent association between specific genetic variations and disease severity was observed. Although different genetic variations among both structural and non-structural genes in each serotype of DF and DHF cases were noted, mutations were not specific for DF or DHF, and did not appear in the DENV strains obtained during subsequent disease outbreaks. (4) All non-dominant genotypes among each serotype had higher rates of amino acid substitutions (2 times more than that of dominant genotypes) among both structural and non-structural genes. (5) Significant inter-serotype diversities were observed; the most conservative serotype is DENV-3, followed by DENV-2, DENV-1 and DENV-4. (6) Diversities among genes in each serotype or genotype were observed; the most conserved gene in DENV-1 is NS2B, in DENV-2 it is NS4B, in DENV-3 it is NS1 and NS2B, in the genotype I of DENV-4 it is NS2B, and in the genotype IIA /III of DENV-4 is PreM/M. (7) Some amino acids pertinent to both structural and non-structural genes in all DENV serotypes were gradually altered, and these mutated amino acids consecutively appeared in subsequent generations.

(ACMCIP Abstract)

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AN INDIVIDUAL BASED MODEL FOR HETEROGENEOUS DENGUE TRANSMISSION INCORPORATING BOTH AGE-DEPENDENT BITING AND SPATIAL HETEROGENEITY

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Most dengue models base transmission parameters on reported clinical cases but for a disease such as dengue where the inapparent to apparent ratio is quite high and is likely modified by various host factors (such as age), transmission rate estimates may be imprecise or even biased. In the relatively few studies where inapparent infections were estimated, the cohort often consisted of a narrow segment of the human population (ie, schoolchildren). However, evidence from mosquito bloodmeal analyses as well as population based prospective serological studies indicates that mosquitoes do not bite humans uniformly, instead they tend to bite adults at a higher frequency than children. Such age-dependent force of infection has implications for vector control and immunization programs because the control effort necessary to interrupt transmission might be greater than would be estimated under a homogeneous biting model. Spatial heterogeneity may also play an important role in dengue virus transmission dynamics because the mosquito vector, *Aedes aegypti*, is a peridomestic species that lives inside houses and rarely travels distances greater than 100 meters. In this study we explore the combination of spatial heterogeneity and age-dependent biting on the dynamics of dengue transmission in an individual-based simulation model. Human and mosquito populations are divided spatially into households. Within households the assumption of homogeneous biting was relaxed to allow for biting rates to vary by human age. Results from this heterogeneous model are compared to a homogeneous model as well as to measurements of actual dengue transmission from a cohort study in Iquitos, Peru and demonstrate the importance of incorporating heterogeneities into models for dengue control because models assuming homogeneity underestimate thresholds such as the critical proportion of the population which must be vaccinated in order to interrupt transmission.

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ARTEMETHER-LUMEFANTRINE VERSUS AMODIAQUINE PLUS SULFADOXINE-PYRIMETHAMINE FOR THE TREATMENT OF UNCOMPLICATED FALCIPARUM MALARIA IN BURKINA FASO

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Drug resistance necessitates new treatments for malaria in Africa. Artemisinin-based combination regimens are widely advocated, but other regimens are cheaper and more readily available. We compared the efficacies of standard doses of artemether-lumefantrine and amodiaquine + sulfadoxine-pyrimethamine to treat uncomplicated malaria in a randomized trial of patients aged six months or greater in Bobo-Dioulasso, Burkina Faso. Primary endpoints were the 28-day risks of treatment failure either unadjusted or adjusted by genotyping to distinguish recrudescence from new infection. Of 521 enrolled patients, 478 (91.7%) completed the 28-day study. The risks of recurrent symptomatic malaria (10.2% versus 1.7%, $p<0.001$) and recurrent parasitemia (15.1% versus 4.7%, $p<0.001$) were greater with artemether-lumefantrine compared to amodiaquine + sulfadoxine-pyrimethamine. Nearly all recurrences were due to new infections. Recrudescences were 4 late treatment failures with artemether-lumefantrine and one early treatment failure with amodiaquine + sulfadoxine-pyrimethamine. Fever clearance was more rapid with amodiaquine + sulfadoxine-pyrimethamine. Hemoglobin increased similarly in the two groups after therapy. Gametocyte carriage was uncommon, and only seen after amodiaquine + sulfadoxine-pyrimethamine. Both regimens were safe and well tolerated, with one serious adverse event in each group, in both cases a drop in hemoglobin to <5 gm/dL. Pruritis was more common with amodiaquine + sulfadoxine-pyrimethamine. Each regimen selected for new isolates with mutations associated with decreased drug susceptibility. The more inexpensive and available regimen, amodiaquine + sulfadoxine-pyrimethamine, offered superior efficacy for the treatment of uncomplicated malaria. For regions of Africa with continued good efficacy of amodiaquine + sulfadoxine-pyrimethamine, blanket recommendations for artemisinin-based combination therapy for the treatment of malaria might be reconsidered.

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ARTEMISININ RESISTANCE ALONG THE THAI-CAMBODIAN BORDER?

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Once it develops and spreads, resistance of *Plasmodium falciparum* parasites to artemisinin derivatives, currently the most essential antimalarial drugs, could have devastating effects on malaria control worldwide. Since 2001, more than forty countries have officially adopted artemisinin-based combination therapy for the treatment of malaria. Recent alarming reports from Southeast Asia suggest increasing numbers of treatment failures even with artemisinin-based combination therapies (ACT). We aimed to investigate reports of increasing numbers of failures with ACT (600 mg of artesunate given over 2 days plus 1250 mg mefloquine) along the Thai-Cambodian border using an integrated *in vitro-in vivo* approach. In a preliminary analysis of parallel *in vitro* and clinical 42-day follow-up data from 42 patients, 28.6% (95% CI: 15.7-44.6%) of the patients were either classified as late clinical or parasitological failure. The 50% inhibitory concentrations (IC_{50}) for dihydroartemisinin in patients who later failed therapy (1.36 ng/ml; 95% CI: 0.89-2.09) were almost double (P=0.028) as high as in patients who did not have recrudescences (0.72 ng/ml; 95% CI: 0.53-0.99). The difference for mefloquine was considerably smaller and not significant (13.03 vs. 10.42 ng/ml; P>0.05) suggesting that mefloquine resistance cannot explain these failures. The pooled artemisinin IC_{50}s were also significantly higher (0.90 vs. 0.53 ng/ml; P=0.001) than those obtained from patients who had acquired their malaria along the western border of Thailand, also an area with high levels of multidrug resistance, but an area where ACT still results in much higher cure rates. Our data suggest that reduced artemisinin susceptibility correlates with ACT failures and that there may be a focus of reduced artemisinin sensitivity leading to high recrudescence rates along the southeastern border with Cambodia. This could indicate an alarming development towards artemisinin resistance that urgently needs further investigation.

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SELECTION OF RESISTANCE-MEDIATING ALLELES AFTER TREATMENT WITH ARTEMISININ-BASED COMBINATION THERAPY IN UGANDA

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Artemisinin-based combination therapy (ACT) is currently advocated in Africa as a means of improving treatment efficacy and slowing the development of drug resistance. However, the selection of resistant parasites, particularly to artemisinin partner drugs, remains a concern. The aim of this study was to assess the selection of alleles in the *Plasmodium falciparum* multi-drug resistance gene *(pfmdr1)* that are associated with decreased sensitivity to the artemisinin partner drugs amodiaquine and lumefantrine. Samples were from a randomized trial of the efficacy of artesunate-amodiaquine and artemether-lumefantrine for the treatment of uncomplicated malaria in Tororo, Uganda, a region of very high malaria transmission. We evaluated polymorphisms of the *pfmdr1* alleles *N86Y, Y184F, S1043C, N1042D,* and *D1246Y* in pretreatment isolates (n=403) and in new infections (n=234) identified by genotyping over 28 days of follow-up. Polymorphisms at the 5 polymorphic *pfmdr1* alleles were assessed by PCR amplification followed by sequence-specific restriction endonuclease digestion. In the artemether-lumefantrine arm, analysis showed selection of wild type alleles in newly infecting parasites (except for *Y184F*): *86N, 8% to 43% (RR=5.2,95%CI 3.1-8.5,p<0.0001); 184F, 4% to 14%(RR=3.6,95% CI 1.6-8.4,p<0.001); 1246D, 12% to 42% (RR=3.4,95%CI 2.2-5.2,p<0.0001).* In contrast, artesunate-amodiaquine showed selection of mutant genotypes in newly infecting parasites: *86Y, 91%(182/201) to 97%(128/132) (p=0.03); 1246Y, 83%(167/201) to 91%(120/132)(p = 0.05): 86Y* and *1246Y, 81.6%(164/201) to 90.2%(119/132) (p=0.04).* For other tested polymorphisms, changes in allele prevalence were not significant. Although artemisinin derivatives are effective in clearing malaria parasites, ACTs select for polymorphisms that may be associated with decreased sensitivity to artemisinin partner drugs, and thus that may alter treatment efficacy.

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THE TRIPLE AND QUADRUPLE MUTANT ALLELES OF *DIHYDROFOLATE REDUCTASE-THYMIDYLATE SYNTHASE* FROM *P. FALCIPARUM* ARE MORE EFFICIENT *IN VITRO* THAN THE WILD TYPE ALLELE

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Studies of *Plasmodium falciparum* in the laboratory and in the field have demonstrated that resistance to pyrimethamine-sulfadoxine (SP) depends on point mutations in the enzyme dihydrofolate reductase (DHFR), the target of pyrimethamine, and dihydropteroate synthase, the target of sulfadoxine. Parasites that carry *dhfr* genes with point mutations at three or four positions (51I/59R/108N or 51I/59R/108N/164L) are resistant to pyrimethamine *in vitro* and patients infected with these parasites respond poorly to SP treatment. The prevalence of these alleles demonstrates their increased fitness over drug-sensitive alleles in the presence of the drug, but an important question remains: Do these mutations that confer drug resistance reduce the fitness of parasites that carry them? The prediction of decreased fitness of parasites that carried the triple and quadruple mutant was supported by early measurements of the DHFR enzyme activity *in vitro*; the catalytic activity of the DHFR domain of both enzymes was severely compromised. We have assayed the enzymatic activity of both resistant alleles in their native configuration as a DHFR-thymidylate synthase dimer purified as a soluble protein from *E.coli.* The K_m values for both mutant enzymes are significantly lower than the wild type ((20.9 uM, triple, 20.7 uM, quadruple, 44.4 uM, wild type,) but the K_{cat} values are about the same (wild type, 1.24/ sec, wild type, 1.23/sec, triple, 1.42/sec, quadruple). Therefore, the enzyme efficiencies, K_{cat}/K_m are significantly better for both mutant enzymes (0.59, triple and 0.69, quadruple) than the wild type (0.28). These data suggest that the success of the *P. falciparum* parasites that carry triple and quadruple mutant alleles may be due both to their antifolate resistance and to the adequate enzymatic activity of the DHFR enzymes they carry.

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ORIGIN AND DISSEMINATION OF CHLOROQUINE-RESISTANT *PFCRT* ALLELES IN ASIA, AFRICA, AND SOUTH AMERICA

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Foci of chloroquine-resistant *Plasmodium falciparum* parasites were first detected in Southeast Asia and South America, and later in Africa. *P. falciparum* chloroquine resistance transporter gene (*pfcrt*, chromosome 7) has been implicated in chloroquine resistance (CQR). The objective of our study was to understand origins and distribution of CQR-associated *pfcrt* alleles worldwide. We investigated *pfcrt* residue 72-76 alleles and flanking microsatellite (MS) haplotypes in *P. falciparum*-infected individuals from Asia (Papua New Guinea [PNG, n = 88, 3 different holoendemic areas], Indonesia [n = 32], India [n = 24]), Africa (Kenya [n = 33], Uganda [n = 29], Ghana [n = 21]), and South America (Brazil [n = 23], Colombia [n = 25], Guyana [n = 17]), using a sequence-specific oligonucleotide probe hybridization assay and multilocus MS genotyping (5 loci, spanning 40 kb). We observed the CQR-associated *pfcrt* residue 72-76 allele S_{agt}VMNT in all samples from PNG and Indonesia, while we observed S_{agt}VMNT in 22/24 and CVIET in 2/24 samples from India. In Africa, we observed CVIET in all samples from Kenya and Uganda, and in 19/21 samples from Ghana; 2 samples from Ghana showed S_{tct}VMNT, confirmed by DNA sequence analysis. In South America, we observed S_{agt}VMNT and/or S_{tct}VMNT in Brazil and Guyana, while CVMET was observed in Colombia. We observed distinct predominant MS haplotypes associated with *pfcrt* alleles in Asia (S_{agt}VMNT = 2, CVIET = 1), Africa (CVIET = 1), and South America (S_{agt}VMNT/S_{tct}VMNT = 1, CVMET = 1). MS haplotype associated with the *pfcrt*-CVIET allele in Indian samples was different from the haplotype observed in Southeast Asian Dd2 and K1 strains and in African samples, suggesting a further differentiation of CVIET allele carrying parasites. Our results suggest that CQR-associated *pfcrt* alleles had at least 2 origins in Asia, one in Africa, and 2 in South America, and that *pfcrt*-S_{agt}VMNT alleles in Asia (India to PNG) have evolved from a common origin, and are genetically different from the *pfcrt*-S_{agt}VMNT allele in South America.

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VARIABLE LENGTH SIMPLE SEQUENCE REPEATS IN PFCRT INTRONS PROVIDE EVIDENCE FOR CONTINUING EVOLUTION FOLLOWING CHLOROQUINE-ASSOCIATED SELECTIVE SWEEPS

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It is hypothesized that selection by chloroquine underlies selective sweeps in *P. falciparum* that have homogenized the pfcrt locus when comparing exon-based single nucleotide polymorphism and microsatellite (MS) markers flanking the gene. Consequently we observe increased linkage disequilibrium across an 40kb region flanking chloroquine resistant (CQR) in contrast to chloroquine sensitive alleles (CQS); linkage disequilibrium decays with increased distance from the gene. Interestingly our recent studies have shown that there is unexpected intron-length polymorphism within pfcrt, specifically at introns 2, 3, and 4. Here we were interested to perform a comprehensive analysis of simple sequence repeats across the total length of the pfcrt genomic sequence. A comparison of published genomic sequence of Dd2 and 3D7 showed that simple sequence/MS repeats (TAn, An, and Tn) were present in all pfcrt introns, with length polymorphisms observed between strains in introns 1, 2, 3, 4, 7, 9, 10, and 11. We have now evaluated all 12 pfcrt introns to characterize length polymorphism in each, for laboratory adapted Pf strains (n=7). Analysis of MS polymorphism was performed by developing fluorescently labeled nested or semi-nested amplification primers for the introns, with at least one of the nest 2 primers annealing to coding sequence conserved between CQS and CQR strains. PCR products were then characterized by PAGE and MS alleles were distinguished based on their relative electrophoretic mobility differences. Length polymorphism was found in all pfcrt introns for the lab strains. Not surprisingly the most polymorphism was present in CQS samples, though multiple introns were polymorphic within both the CQR-associated pfcrt-SVMNT and CVIET groups. The polymorphism within these intronic sequences suggests that the pfcrt gene sequence is undergoing continued evolution. The mechanism responsible for this variation may involve DNA polymerase slippage across AT-rich sequences and/or inter-strain gene conversion through recombination.

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PFNHE POLYMORPHISMS AND CLINICAL QUININE RESISTANCE IN MALI

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Quinine is the drug recommended for treatment of severe malaria in most sub-Saharan countries. The mechanism of *Plasmodium falciparum* resistance to quinine is not known. Recent QTL mapping studies indicate that genetic loci on several chromosomes may be involved, including polymorphisms in a *P. falciparum* Sodium-Hydrogen Exchanger (PfNHE) on chromosome 13. To investigate the role of those polymorphisms in malaria endemic settings we are conducting prospective *in vivo* quinine efficacy studies in Kolle, Mali where malaria is hyperendemic with seasonal peaks. Between September and December 2005 consenting cases of non-*per os* malaria were included, treated with standard doses of quinine for 5 days and followed up for 28 days. Treatment outcome (early treatment failure, ETF, late clinical failure, LCF, late parasitological failure, LPF and adequate clinical and parasitological response, ACPR) were classified using modifications of the World Health Organisation's 2003 protocol for the assessment of antimalarial efficacy. Molecular markers of parasite polymorphisms msp1, msp2 and the microsatellite CA1 were PCR amplified to distinguish recrudescent parasites from new infections. Overall 48 patients were included with one case of withdrawal. Raw *in vivo* quinine efficacy showed 0%, 12.8%, 46.8% and 40.4% of ETF, LCF, LPF and ACPR, respectively. After molecular correction, 100% of the infections were ACPRs. In vitro quinine efficacy studies on these parasites are underway. Prevalence of polymorphisms of the PfNHE microsatellite MS4760.1 in post-treatment parasites will be presented. This study is the first investigation of quinine clinical efficacy in Mali. Implications for the management of severe malaria and mechanisms of quinine resistance will be discussed.

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A LONGITUDINAL STUDY OF *CRYPTOSPORIDIUM* INFECTION IN CHILDREN IN DHAKA: THE ROLE OF GENETIC SUSCEPTIBILITY TO INFECTION

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Diarrheal disease from cryptosporidiosis is common and can be protracted and severe in infants and immunocompromised individuals. 226 preschool and school-aged children were prospectively followed for over three years to study the community impact of *Cryptosporidium* and the role of host genetics in susceptibility to infection. Epidemiologic and clinical data were captured, as well as diarrheal and surveillance stool specimens and DNA for genetic analysis. HLA class I and II polymorphisms were analyzed by PCR using sequence-specific oligonucleotides. Single nucleotide polymorphisms (SNPs) in the genes of IL-8, TNF- RI, IL-12p40, TLR-5 and TLR-2 were tested by PCR. Ninety-six children (42.6%) were diagnosed with *Cryptosporidium*, a total of 142 distinct episodes. Fifty-eight (25.7%) children had cryptosporidiosis (*Cryptosporidium* diarrhea), 51 (22.6%) had asymptomatic infection and 17 children had recurrent cryptosporidiosis (29.3%). 75% of children with cryptosporidiosis presented on the first day of illness, and most had abdominal pain (58%) and a short course of diarrhea (mean, 3 days). Genetic analysis revealed no associations between *Cryptosporidium* infection and HLA class I alleles or functional SNPs in IL-8, TNF R1, IL-12p40, TLR-5 or TLR-2 genes. In contrast, children with *Cryptosporidium* infection were nearly 3 times more likely to carry the HLA class II DQB1 *0301 allele (OR = 2.75, p value= 0.005) vs. children without infection. Although the numbers of children per category were small, a strong association was found with *Cryptosporidium* asymptomatic and symptomatic children carrying 2 copies of the DQB1 *0301/ DRB1 *1101 haplotype (OR=7.02, p value 0.008) vs. children with no infection during the 3 years of follow-up. We conclude that cryptosporidiosis in Bangladeshi school-aged and preschool children is common, and often recurrent, but of shorter duration and characterized by more abdominal pain than in infants. Consistent with the role of cell-mediated immunity and CD4+ T cells in the resolution of cryptosporidiosis, *Cryptosporidium* infection is associated with the HLA class II allele *0301 and may also be associated with the homozygous DQB1 0301/ DRB1 *1101 haplotype. No association of *Cryptosporidium* with cytokine or TLR gene mutations was found, but more complete gene analyses are needed.

(ACMCIP Abstract)

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CONFIRMATION OF ZOONOTIC TRANSMISSION OF *ENTEROCYTOZOON BIENEUSI*

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Enterocytozoon bieneusi causes diarrhea in immunocompromised individuals and also infects immunocompetent people. Phylogenetic studies have suggested that zoonotic *E. bieneusi* transmission can occur, as several genotypes have been reported in both humans and animals. Nonetheless, all these are based on comparison of *E. bieneusi* DNA sequences from unrelated cases. In this study, we conducted a prospective pediatric cohort study of enteric protozoa in Pampas de San Juan, Peru, from March 2002 to March 2006. Upon detection of a case, we also requested stool samples from people and animals living in the household. Stool specimens were initially screened for microsporidia by microscopy using the Weber modified trichrome stain. Positive samples were further characterized using a genotyping tool based on the ITS region of the rRNA gene. In May 2005, we identified a household where a cohort participant and a guinea pig were positive for microsporidia. A total of 6 guinea pigs from this household were positive by microscopy, while the positive person had 3 positive stools. Samples collected over the next three weeks revealed that the case-participant had cleared the infection, while the guinea pigs still had intermittent spore shedding. DNA was extracted for confirmation of the microsporidiosis diagnosis and genotyping of *E. bieneusi*. All microscopy-positive samples from the study participant and 5 of the 6 guinea pigs in the household were positive by PCR. Sequence analysis revealed that the human and all guinea pigs were infected with a genotype that is genetically distant from other genotypes found in humans and appears to be unique to guinea pigs. This is the first documentation of zoonotic transmission of *E. bieneusi*.

(ACMCIP Abstract)

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MICROSPORIDIA SPECIES KNOWN TO INFECT HUMANS ARE PRESENT IN AQUATIC BIRDS; IMPLICATIONS FOR TRANSMISSION VIA WATER?

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Human microsporidiosis, a serious disease of immunocompetent and immunosuppressed people, can be due to zoonotic and environmental transmission of microsporidian spores. A survey utilizing conventional and molecular techniques for examining feces from 570 free-ranging, captive, and livestock birds demonstrated that a total of 21 animals shed microsporidian spores of species known to infect humans, i.e, *Encephalitozoon hellem* (20 birds; 3.5%), and *E. intestinalis* (1 bird; 0.2%). Of 11 avian species that shed *E. hellem* and *E. intestinalis*, 8 represented aquatic birds, i.e., common waterfowl. The prevalence of microsporidial infections in waterfowl (i.e., 8.6%) was significantly higher than in other birds (1.1%) (P<0.03), waterfowl fecal droppings contained significantly more spores (mean: 3.6 x 10^5/g) than non-aquatic bird droppings (mean 4.4 x 10^4/g) (P < 0.003), and positivity for human-infectious microsporidian spores in fecal samples was statistically associated with the aquatic status of the avian host (P<0.001). It was demonstrated that a single visitation of waterfowl flock can introduce into the surface water approximately 9.1 x 10^8 microsporidian spores of species known to infect humans. The study demonstrates that waterborne microsporidian spores of species that are infectious to people can originate from common waterfowl, birds that usually occur in large numbers and have unlimited access to surface waters including waters used for drinking water production.

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CATS AND THEIR FECES: A PROBLEM FOR PUBLIC HEALTH AND WILDLIFE

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The popularity of cats (*Felis catus*) as pets in Europe and the United States, and the movement to protect feral cats has lead to increasing concern about the impact of free-roaming cats on wildlife and the environment. Although negative effects on habitat have been most frequently attributed to feral cats, the high proportion of owned cats allowed outdoors suggests that they too have ample opportunity to defecate in the environment. This finding is relevant when considering the potential impact of zoonotic pathogens shed in feline feces, such as *Campylobacter* and *Salmonella* spp., ascarids (e.g. *Toxocara cati*), hookworms (*Ancylostoma* spp.), and protozoan parasites such as *Toxoplasma gondii.* Our research shows that cat feces are contaminating coastal areas, allowing for waterborne transport of *T. gondii.* The environmentally-resistant oocyst stage of this pathogen is shed in cat feces and is a major cause of mortality in southern sea otters off California's coast. A telephone survey was conducted in a central California coastal community to evaluate how cats were managed and to determine the magnitude of outdoor fecal deposition by cats. A high proportion (44%) of owned cats defecated outside > 75% of the time. Outdoor fecal deposition (wet weight) amounted to 107.6 (metric) tonnes/year in a community of 29.4 km^2, and owned cats accounted for 72% of the estimated tonnage. Seroprevalence and fecal surveys of cats from the area provided a means to estimate the number of oocysts entering the nearshore ecosystem, and will be incorporated as one of the components in a model to estimate the risk for toxoplasmosis in sea otters.

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MICROARRAY-MEDIATED TRANSCRIPTOME COMPARISON OF ENTAMOEBA HISTOLYTICA TROPHOZOITES *IN VIVO* AND *IN VITRO*

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The early branching eukaryote *Entamoeba histolytica* colonizes and invades the human colon. Growth of *E. histolytica* trophozoites in the gut may depend on the expression of key metabolic enzymes, proteins or pathways. Regulation of mRNA abundance can modify cellular metabolism in response to environmental cues. Microarray analysis was performed to compare the transcriptome of *E. histolytica* isolated from the colon of five infected mice and the transcriptome of ameba cultured *in vitro*. Adaptation to the intestinal environment was associated with significant decreases in mRNA abundance for genes involved in glycolysis and concomitant increases in lipases. This is consistent with a change in energy metabolism. As expected in the anaerobic colonic lumen there were significant decreases in transcripts involved in oxygen detoxification pathways. A subset of the mRNAs encoding cell signaling genes including transmembrane kinases, ras and rho family GTPases, and calcium binding proteins were changed, suggesting that these genes could be involved in the interaction with the host environment. The changed mRNA levels of genes similar to the AIG1 plant antibacterial proteins may indicate a possible response to the presence of the colonic bacteria. Of the known virulence factors, a cysteine proteinase 4-like transcript was increased 20-35 fold and two members of the Gal/GalNAc lectin light subunit family showed a 2-3 fold decrease. The intestinal transcription signature of *E. histolytica* may be partly due to the initial modulation of several potential *E. histolytica* transcription factors. This analysis has identified the enzymes involved in oxygen defense, the AIG family and several proteins involved in both DNA and phosphate signaling as potential new virulence factors, not previously identified through the use of *in vitro* cultures.

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A MULTIPLEX REAL-TIME PCR ASSAY FOR SIMULTANEOUS DETECTION OF FREE-LIVING AMEBAS IN CLINICAL SPECIMENS

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Infections by free-living amebas occur throughout the world and pose many diagnostic challenges. To date, at least 440 cases of severe central nervous system (CNS) infections by *Naegleria fowleri*, *Acanthamoeba* spp. and *Balamuthia mandrillaris* have been documented worldwide. Most of these cases were fatal. In addition, *Sappinia diploidea* was recently indicated as the causative agent in a fatal case of amebic encephalitis. Rapid and specific identification of these free-living amebas in clinical samples is of crucial importance for efficient case management. We have developed a triplex real-time PCR assay that can simultaneously identify *Acanthamoeba* spp., *B. mandrillaris* and *N. fowleri* in the same PCR reaction vessel. The assay is based on TaqMan probes that target species-specific regions of the chromosomal 18S rRNA gene. The assay was validated with 23 well-characterized amebic strains harvested from cultures and 31 clinical CNS specimens, of which 18 were unfixed archived cerebrospinal fluids that had been stored frozen for up to 29 years. The assay demonstrated high specificity and a rapid test completion time of less than 5 hours from the reception of the specimen in the laboratory. The limit of detection was one ameba per sample analyzed, as determined with cerebrospinal fluid spiked with diluted cultured amebas. This assay could become useful for fast laboratory diagnostic assessment of free-living amebic infections in laboratories with adequate infrastructure to perform real-time PCR testing. It could also be used for retrospective studies of archived samples, since storage did not seem to impair the molecular identification. Furthermore, we have initiated studies on the 18S rRNA gene from *Sappinia diploidea*, with the aim of including the detection of this pathogen in the multiplex real-time PCR assay described here.

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PATHOGENIC PROTOZOA IN SAF-PRESERVED STOOL : RESULT REPRODUCIBILITY AND SPECIMEN DETERIORATION

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Efficiencies in the parasite laboratory must take specimen quality, time delays, storage conditions and other factors into account. The objective of this study was to determine reproducibility of stool O&P exams by comparing results from an original specimen with results from re-submitted specimens (two single and one pooled specimen, submitted

between 2 and 8 weeks later). Patients routinely submit 2 stool specimens to the parasitology laboratory of the McGill University Tropical Disease Centre. Specimens are stored in SAF (sodium-acetate-formalin) until examined within 2-10 days. Concentration and iron-hematoxylin stain are performed. As part of an ongoing quality assurance program, those stool specimens positive for pathogenic and non-pathogenic protozoa, with sufficient fecal material to make up additional (diluted 25-33% from the original to maintain standard volume) specimens (for two single and one pooled specimen re-submissions), were eligible for this study. These additional specimens were accessioned, under false names, as new specimens. We report on results obtained for the pathogenic protozoa (*Entamoeba histolytica/dispar*, *Giardia intestinalis* and *Dientamoeba fragilis).* Of a total of 36 specimens positive for *E. histolytica/dispar*, 32 (88.9%) were identified on the first submission with 26 (72.2%) and 27 (75.0%) identified on re-submission of single and pooled specimens, respectively. Of a total of 21 *Giardia*-positive specimens, 100% were identified originally, with only 7 (33.3%) identified from re-submitted single specimens and 7 (33.3%) from pooled specimens. Of a total of 23 *D. fragilis*-positive specimens, 19 were identified on first examination with only 11 (47.8%) and 14 (60.9%) identified from re-submitted specimens, respectively. Our results suggest a deterioration in the quality of stool specimens over time in terms of detection of low levels of the pathogenic flagellate protozoa. This has important implications for both quality control and quality assurance programs.

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WHERE ARE THE TAPEWORMS?

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My research group has been evaluating alternatives for controlling cysticercosis. With Marshall Lightowlers recombinant vaccines were evaluated. For challenge of pigs we had to obtain fresh *Taenia solium* eggs, one field trip to a rural community in Yucatan, with risk factors associated to cysticercosis, was organized by Rosanna Rodriguez-Canul in November 2003, 450 fecal samples were analyzed for coproantigen (CpAg) ELISA. Beginning January, cestocidal treatment followed by Epsom salt were administered to 21 CpAg positive, no tapeworms were recovered. In a second trip in April 2004 to 2 rural communities in Oaxaca with similar conditions, we analyzed 200 fecal samples by CpAg, cestocidal treatment followed by magnesium milk were administered the next day to 20 positive people. Again, no tapeworms were recovered. To search for explanations to our failure we analyzed community based data published by various authors in the last 15 years, and found that: 1) Prevalence by CpAg or coproparasitoscopic studies in Mexico is 1.2% while in Peru is 2.5% and in Guatemala is 2.7%. 2) In Mexico tapeworms were recovered from around 37% of carriers detected by CpAg and from 66% of those that indicated having released proglottids after showing them pictures of tapeworms or fixed parasites; this latter approach increased in one year notification of taeniosis 6 times in an endemic jurisdiction of over 700,000 inhabitants. 3) In Peru the evaluation of purgatives in parasitological confirmed cases from hospitals and communities showed that gravid proglottids were expelled in 34% and 41% patients with castor oil and in 53% and 69% with polyethylene glycol salt, respectively. Therefore several questions should be analyzed: 1) Is the incidence of taeniosis in Mexico very low to be detected in asymptomatic carriers? 2) Has ELISA low sensitivity? 3) Have drugs and purgatives low efficiency? 4) Do tapeworms live in their hosts for short periods and are released spontaneously? 5) Are there biological factors of *Taenia solium* which are still unknown?

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TAENIA SOLIUM ONCOSPHERE *IN VITRO* ADHERENCE

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The specific mechanisms underlying *Taenia solium* oncosphere adherence and penetration in the host have not been studied yet. We developed an *in vitro* model adhesión assay to evaluate the mechanism of adherence of *T. solium* oncospheres to the host cell. We used monolayer Chinese hamster ovary cells (CHO) to determine the optimum time of oncosphere incubation, the role of sera and the effect of temperature on oncosphere adherence. Using light and scanning microscopy we observed the morphologic characteristics of adhered oncosphere. Oncosphere adherence was significantly greater enhanced by fetal bovine sera at 5% or more concentration than without sera ($p<0.05$). The incubation of those oncospheres at 4°C decreased the adherence as compared to 37°C. This would be also the first report demonstrating that *T. solium* oncospheres attach to cells through microfibril processes and expel external secretory vesicles which have the same oncosphere membrane.

(ACMCIP Abstract)

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COMPARISON OF ANTI-PARASITIC REGIMENS FOR PORCINE CYSTICERCOSIS

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Neurocysticercosis (NCC), the infection of the central nervous system by the larval stage of the *Tenia solium,* is a major public health problem in most developing countries. Albendazole (ABZ) and Praziquantel (PZQ) have been used in humans since 1979, and their efficacy to kill parasites has been well demonstrated in humans and pigs. Published data consistently shows 30-40% of treated individuals become free of brain viable cysts, and 70-80% of cysts disappear after one course of anti-parasitic treatment. We evaluated the efficacy of different drugs in pigs naturally infected with cysticercosis. Pigs (n=6 per group) received either PZQ (three 25 mg/k doses in a single day), ABZ (15 mg/k/d for 7 days), PZQ plus ABZ (doses as above), PZQ plus reduced ABZ (7.5 mg/k/d, 7 days) and Oxfendazole (a single dose of 30 mg/k). All ABZ treatments were given with prednisone. A comparison group received prednisone only (0.5 mg/kg, 7 days), and a second comparison group was left untreated (negative control). Animals were sacrificed ten weeks after treatment, and the whole carcasses were exhaustively examined. The numbers of viable cysts in muscle were significantly decreased in pigs receiving oxfendazole, ABZ, or ABZ+PZQ. Pigs receiving PZQ alone had numerous surviving cysts. For brain cysts, the combination of ABZ+PZQ was more effective than ABZ given alone, while PZQ and oxfendazole left many viable cysts. Controls (untreated) pigs and prednisone-treated pigs showed no effect on cyst numbers in muscle or brain. Combination therapy may increase parasiticidal efficacy for treatment of human neurocysticercosis.

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NEUROCYSTICERCOSIS - FAST INFLAMMATORY RESPONSE IN PIG BRAINS FOLLOWING ONSET OF TREATMENT WITH PRAZIQUANTEL OR ALBENDAZOLE

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Neurocysticercosis is one of the most common parasitic diseases of the nervous system. Medical management involves the use of anti-parasitic drugs, which frequently exacerbate the patient's symptoms by triggering an inflammatory response of the host to antigens exposed by the attacked parasites. This pilot study assessed whether this inflammatory reaction in natural host tissues could be detected in the initial 12 to 48 hours after onset of treatment with albendazole or praziquantel, and whether it had similar characteristics for both drugs. Fifteen naturally infected pigs were bought and allocated to 5 groups of three pigs each. The control group received no treatment. Six pigs received 50mg/Kg of praziquantel administered in 3 doses every 8 hours, and the remaining 6 pigs received 15 mg/Kg albendazole administered in 3 doses every 12 hours. For both PZQ and ABZ treated animals, three animals were sacrificed 12 hours after treatment onset and the other three were sacrificed 48 hours after treatment onset. At necropsy, cysts were obtained from muscle and brain, fixed in 10% PBS-formaline, and stained with H-E. The inflammatory reaction was assessed in terms of quantity and extension, and type of cells. Pigs sacrificed at 12 hours after treatment showed a mild inflammatory reaction, represented by the presence of eosinophils and lymphocytes. Comparatively, this reaction was higher and more structured in the ABZ group. At 48 hours the degree of inflammation was more marked, again comparatively stronger and more structured in the ABZ group in which a well defined layer of eosinophils could be clearly seen in most samples. The cellular reaction was stronger in muscle than in brain in all groups included the control group. The inflammatory response which follows anti-parasitic treatment of cysticercosis occurs much earlier than expected, and seems to follow different patterns in pigs treated with albendazole than in those treated with praziquantel.

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PIG MODEL TO IDENTIFY AND QUANTIFY CALCIFICATION PROCESS OF CYSTICERCOSIS LESIONS

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Taenia solium neurocysticercosis is the single major cause of acquired epileptic seizures in the world, and a common diagnosis in immigrant populations in the USA and other industrialized countries. Intraparenchymal NCC (viable cyst, degenerating cyst and calcified lesions) causes epilepsy or focal neurological problems. The death of the cyst is associated with a scarring and calcification process. This study was intended to explore the feasibility of using infected pigs as a model to detect and measure calcium deposit during the calcification process of parasites in brain and muscles. We examined archived paraffin-embedded samples of naturally infected pigs which had been treated with antiparasitic drugs and sacrificed 10 weeks after. Two techniques were used to stain calcium, Alizarin red S and Von Kossa. The lesions were macroscopically identified during necropsy and preliminary confirmed with Hematoxylin-Eosin staining as cystic lesions, degenerating cysts, degenerating lesion with calcification, or calcified lesions. The slides were read by two pathologists and an image analysis system will used to quantify the lesion area. Results show that calcium can be identified in this sort of lesions by both Alizarin red S and Von Kossa stains. In the viable stage, minimal amounts of calcium can be detected in the calcareous corpuscles. From then on, the calcium concentration increase progressively according to the macroscopic degree of degeneration of the lesions (degenerating cysts, degenerating lesion with calcification, and calcified cysts). The calcification process in muscles is faster and more prominent than in brain. This model could potentially open a significant and novel approach to understand and test the therapeutic approach in the calcification process.

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NEUROCYSTICERCOSIS IN *T. SOLIUM* TAPEWORM CARRIERS

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Human taeniasis caused by *Taenia solium* is usually asymptomatic; nevertheless it would be the only source of infection with the larval form, cysticercosis. Mainly due to the low prevalence of infection, no estimates of the risk of neurocysticercosis in tapeworm carriers are available. This study included 79 *T. solium* tapeworm carriers diagnosed by stool examination or coproantigen detection and confirmed by morphology or PCR. These patients also had a CT scan or MRI performed, because of participation in epidemiological research studies or by medical indication. Most neurologically symptomatic patients found to carry a tapeworm had cysticercosis-compatible images on CT or MRI (29/31, 94%): 68% viable cysts, 13% granulomas, 13% calcifications. The compromise of the nervous system was less frequent in asymptomatic carriers. Brain calcifications were found in 2/11 (18%) of asymptomatic housemaids who carried a *T. solium* tapeworm (working in Lima but coming from endemic areas); as well as in 13/37 (35%) of asymptomatic *T. solium* carriers detected in field surveys. As a reference, the prevalence of NCC diagnosed by images in endemic areas varies between 8 to 20%. As expected, tapeworm carriers with neurologic symptoms had more frequent compromise of the CNS, and harbored live parasites in a significant proportion of cases. Asymptomatic tapeworm carriers from endemic areas had brain calcifications with a higher frequency than the rest of the population living in the same area, and apparently those who migrate to non endemic areas have calcifications in a lower proportion of cases (perhaps because of improved sanitary conditions).

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BRAIN CALCIFICATIONS IN 26% OF GENERAL POPULATION IN A CYSTICERCOSIS-ENDEMIC VILLAGE IN TUMBES, PERU

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A high frequency (8~18%) of intraparenchymal brain calcifications has been reported from studies using computed tomography in samples of general population in cysticercosis-endemic areas. As part of a wide control program evaluating different intervention measures we asked the entire adult population of an endemic village to have a non-contrasted brain CT scan performed and a blood sample for detection of cysticercosis antibodies. From 269 adults, 259 accepted to participate in the study. The prevalence of antibodies to cysticercosis on Enzyme-linked immunoelectrotransfer blot assay was 36%. From 204 brain CT scans, one or more round, intraparenchymal punctuate calcifications were found in 53 persons (26%). Conversely, brain lesions compatible with viable cysts were found in only two individuals. There was an association between seropositivity and a positive CT scan. Most of these 53 individuals were apparently neurologically asymptomatic. Intraparenchymal brain calcifications are an extremely frequent finding in cysticercosis-endemic areas and are associated with neurological morbidity in a proportion of the affected individuals. Current information does not allow to assessment of sub-clinical morbidity.

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DATHOXIN-1, A DUAL FUNCTIONAL AND BLOOD FEEDING REGULATED SALIVARY GLAND PROTEIN OF THE TICK, *DERMACENTOR* AND*ERSONI*

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Salivary gland gene expression and saliva protein composition change during the course of blood feeding. Tick saliva contains pharmacologically active molecules that inhibit host hemostasis, pain/itch and immune defenses. We report identification and characterization of a novel salivary gland bioactive molecule, dathoxin-1, with both anti-coagulant and anti-inflammatory properties. Dathoxin-1 was cloned from a cDNA library prepared from 18-24 hour fed salivary glands of D. andersoni females. This cDNA library contained 16 putative protease inhibitor clones with one or two Kunitz domains. Combined use of 5′ and 3′ RACE strategies resulted in a full length sequence of 618 nucleotides, which was confirmed by Northern blot analysis. Recombinant dathoxin-1 expressed in High Five™, Trichoplusia ni, insect cells inhibited the intrinsic coagulation pathway and trypsin in a dose-dependent manner. A quartz crystal microbalance method was used with recombinant dathoxin-1 as bait to identify specific binding activity for the coagulation factor XIIa. Dathoxin-1 is a member of a family of four genes, dathoxin-1 through 4, which are up-regulated by blood feeding and each encode two Kunitz domains. Quantitative real-time RT-PCR revealed differential expression patterns of these four genes during blood feeding. Dathoxin-1 expression peaked during the second day of feeding with a 174.7 $\pm$ 51.19 fold increase and then decreased to low levels of expression on days three through seven. Expression of dathoxin-3 gene increased on day two and remained elevated through day six of feeding. Expression of Dathoxin-2 and 4 was lower than that of the other two genes with highest expression of dathoxin-4 on days 4 - 6 of feeding. These results identify a novel regulator of host hemostasis and possibly immune defenses and demonstrate that expression of tick genes vary over the course of feeding within a family of closely related genes.

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NOVEL METHODS FOR ADULT AND IMMATURE SAND FLY CONTROL -- PHASE I, LABORATORY TRIALS

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Leishmaniasis is a significant problem affecting civilians and military personnel stationed in endemic areas worldwide. Control of vector sand flies (SFs) and the prevention of leishmaniasis has not been successful using the current methods. Innovative methods are needed to break the transmission cycle by targeting the SF-rodent reservoir portion of the *Leishmania* cycle. Our goal is to control leishmaniasis by precision targeting of rodent reservoirs, using systemic endectocides for unique SF control methods. The sand rat (*Psammomys obesus*), a major reservoir host in Israel, Iraq, and other Middle Eastern and North African countries, were fed endectocides (ivermectin or doramectin) in their diet. After 3 weeks of treatment, they were challenged periodically with naïve, uninfected SFs periodically over 10 weeks. For each treatment group, adult SF mortality, fecundity, egg hatch, immature SF development, and F1 adult emergence were recorded. Feces with residual endectocides were fed to naïve larval SF in order to determine larval control efficacy and persistence of the insecticides. Both ivermectin and doramectin caused significant decreases (90-95%) in the number of eggs laid with no survival of 1st instar larva at 8 days post emergence. The results and future field testing will be discussed.

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PRELIMINARY STUDIES ON THE FEEDING RELATIONSHIPS WITHIN MACROINVERTEBRATES IN WATER BODIES ASSOCIATED WITH *MYCOBACTERIUM ULCERANS* DISEASE TRANSMISSION

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Buruli ulcer (BU), a debilitating disease of the skin, is caused by an aquatic environmental saprophytic mycobacterium, *Mycobacterium ulcerans*. The pathogen has been detected in human biting aquatic hemipterans of the families Naucoridae and Belostomatidae suggesting aquatic insect bites as a possible mode of infection to man. The present study is being conducted to elucidate feeding relationships within macro invertebrates in water bodies associated with BU endemic communities as a basis of identifying aquatic invertebrates that by their feeding habits could be implicated in the proliferation and transmission of *Mycobacterium ulcerans.* The preliminary study was conducted in the Amansie West and Ga Districts in Ghana. From the 15 water bodies sampled, 44 macro invertebrate families belonging to 17 orders and 5 classes were identified. Majority were insects (85%) and 59% belonged to the predator (engulfers and piercers) functional feeding group. Others were scrapers (19%), filterers (12%), shredders (4%) and suckers (2%). Belostomatids identified were, *Diplonychus* sp, *Appasus nepoides* and *Limnogeton fieberi* and for Naucoridae, *Macrocoris* sp and *Naucoris* sp. The feeding relationships implicate all human biting aquatic macro invertebrates (Families Nepidae, Notonectidae and Corixidae) as potential agents of transmission of the pathogen to man. Shredders, filterers and scrapers (primary consumers)

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which together formed 33% of invertebrate families are being examined for *M ulcerans* since they may form the primary entry group of the pathogen into the aquatic macro invertebrate feeding web.

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ASSESSEMENT OF TRIATOMA DIMIDIATA DISPERSAL IN THE YUCATAN PENINSULA OF MEXICO USING MORPHOMETRY AND MICROSATELLITE MARKERS

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In the Yucatán peninsula, Mexico, the main vector of Chagas disease is *Triatoma dimidiata*, an apparently ubiquitous species of triatomine. Field studies suggested that natural transmission would occur through the transient and seasonal invasion of houses by sylvatic triatomines, rather than through domiciliated bug populations. We thus investigated the genetic structure of *T. dimidiata* populations, using morphometry and microsatellite markers, to assess dispersal of this triatomine species and understand domicile infestation. We observed a low phenotypic and genetic differentiation between populations from different villages suggesting a weak but significant population structure at this level, with Fst ranging from 0.001 up to 0.049. A similarly low but significant difference was observed between populations from the same villages but different biotopes (sylvatic, peridomestic and domestic), with Fst ranging from 0.010 to 0.046. These data suggested a rather elevated gene flow between biotopes (Nm = 5-6), which was confirmed by likelihood and bayesian assignment tests. Indeed, an important proportion of bugs collected in the domiciles were significantly assigned to peridomestic and sylvatic areas. This study thus indicates that *T. dimidiata* has important dispersal capabilities that can explain the seasonal pattern of domicile infestation by peridomestic and sylvatic bugs. This dispersal needs to be taken into account for the design of effective vector control strategies.

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ESTABLISHMENT OF BASELINE PYRETHROID SUSCEPTIBILITY LEVELS IN THE MAIN MALARIA VECTORS IN GHANA

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The use of insecticide treated nets (ITNs) is a key component in the strategy for malaria control in Ghana. However, existing pyrethroid resistance in mosquito populations before ITNs intervention may render them ineffective. Very little information is available on the susceptibility status of *Anopheles* malaria vectors in Ghana. Therefore, there is a need of establishing insecticide susceptibility levels before introducing ITNs over a wider area. The study was set to generate such data and frequency distribution of *kdr* allele. From January to December 2005, blood-fed *An. gambiae* s.l. mosquitoes were collected from 10 localities in 5 regions of Southern Ghana reared to F1 for bioassay tests. Bioassays yielded the knockdown (KD) of wild *An. gambiae* ranging from 68% to 98%, 4% to 99% and 4% to 82% for deltamethrin, permethrin and DDT respectively. Mortalities were ranging from 86% to 99%, 34% to 96% and 5.7% to 93.5% for deltamethrin, permethrin and DDT respectively. KD and mortality rates for Kisumu reference strain of *An. gambiae* s.s. were consistently 100%. PCR test for 545 *An. gambiae* s.l. revealed that *An. gambiae* ss was the only sibling species occurring in 9 localities and counted for 90% in the other locality where it was sympatric with *An. melas* (10%). Of 445 *An. gambiae* identified to molecular forms, 75.51% were S-form and 24.49% M-form. They were sympatric in all localities but no S/M hybrids were detected. The frequency of *kdr* allele was 45.17% and 8.99% for S and M-forms respectively.

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INTERLEUKIN-1 RECEPTOR ANTAGONIST (IL1RA) POLYMORPHISMS AND DIARRHEA OUTCOMES IN BRAZILIAN SHANTYTOWN CHILDREN.

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Interleukin-1 receptor antagonist gene (IL1RN) polymorphisms, which are distinguishable by the number of 86bp tandem repeats in intron 2, have been widely studied. Previous groups have shown that, allele 1 has the highest frequency (73.6%), followed by allele 2 (IL1Ra*2, 21.4%) in the general population. Moreover, many groups have shown that allele IL1Ra*2 is associated with a number of human diseases, such as gastric cancer or lupus. In these regards, the IL1Ra*2 has been identified as "proinflammatory," (Omar et al, 2000). Despite these correlations with deleterious inflammation and disease, more recent studies suggest that serum IL1Ra could also play a positive role, since IL1Ra seems to protect children with leukemia from overt malnutrition (Schmid et al, 2005) and a Korean group found that IL1Ra*2 was significantly linked to obesity (J.-Y. UM et al, 2006). In this study, we performed IL1Ra genotyping on buccal cell DNA collected from 130 shantytown children in Fortaleza, Ceará, in Northeast Brazil, where malnutrition caused by diarrhea is common. Quantitative ELISA assays for lactoferrin on fecal specimens from 51 out of the 130 children were also performed. IL1RN polymorphisms and fecal lactoferrin concentrations were then integrated into our previous long-term cohort study database and analyzed with SPSS. IL1RN allele frequencies among favela children are close to those in European Caucasian populations (30% and 21-23% respectively), where it has been linked to gastric cancer and autoimmune disorders. However, IL1Ra*2 may play a protective role against diarrhea outcomes in poorer populations. Preliminary analyses of lactoferrin results showed trends that IL1Ra 1/2 and 2/2 genotypes are associated with higher fecal lactoferrin concentrations in acute diarrhea stools when compared with genotype 1/1 (p=.195 and p=.294). At the same time, IL1Ra*2 was significantly associated with higher HAZ scores at 12 months of age and WAZ scores at both 12 and 24 months of age (p=0.043, p=<0.001, and p=<0.001, respectively, Linear-by-Linear Association) among children with high diarrheal burdens. In conclusion, here we show novel findings of IL-1Ra polymorphisms during children's growth and development and diarrhea outcomes in disenfranchised populations.

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RECONTAMINATION OF HOUSEHOLD DRINKING WATER: A CONTROLLED EXPERIMENT IN NORTHERN COASTAL ECUADOR

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Waterborne diseases are still responsible for approximately 1.8 million deaths each year. If targets such as the Millennium Development Goal of halving the number of people without access to safe water by 2015 are to be reached, high quality research is needed to assess how to most effectively address problems of drinking water quality. Many researchers have shown a tendency for household water samples to be more contaminated than the source waters from which they draw, but few if any researchers have carried out paired samples to show definitively that water is contaminated in the household. We describe the results of a controlled experiment to assess contamination of drinking water

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between the source and point-of-use in northern coastal Ecuador. These villages rely on untreated surface water and simple piped water systems for their drinking water. Source water samples were taken at the time that household members filled their drinking water containers, and a control container was filled at the same time and kept in controlled conditions to avoid recontamination. Household and control containers were then resampled daily until the household water was finished to evaluate water quality as measured by a suite of indicator organisms including E. coli, Enterococci, and coliphage. This experimental design allows for a controlled assessment of die-off and recontamination events, comparing source waters to both control and household samples. To our knowledge this is the first study to use paired samples or controls in assessing recontamination between source and point-of-use drinking water quality.

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PRELIMINARY INVESTIGATIONS OF HOST-SEROVAR SPECIFICTY AND INFECTION PREVALENCE OF PATHOGENIC LEPTOSPIRES IN HAWAIIAN RODENTS

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Hawaiian watersheds are discrete mountain-to-sea ecological regions that offer a unique study system in which to investigate leptospirosis, a pantropical zoonotic disease with the highest national mean annual incidence in Hawaii. To assess past trends of pathogen transmission, we studied a disease surveillance database spanning 1992 to 2003 and encompassing major islands of the Hawaiian Islands chain. On the island of Hawaii, annual prevalence was found to be correlated between host species, particularly the black rat and house mouse (r^2=0.94, t = 6.58, p <.001) and the black rat and Indian mongoose (r^2=0.78, t = 3.05, p =.02). The synchronicity of these two species with rats and the high peaks for 3-4 species in 1994 and 1997 suggest that these species may be affected by a common extrinsic factor such as rainfall. In fact, in a logistic regression, rainfall explains a large amount of the variance in prevalence ($F_{(33, 34)}$ = 8.52, p < 0.0063), second only to host species. Together these preliminary data indicate varying degrees of inter-annual variation and shifting host use patterns. We also present results of a follow-up host-serotype distribution studies recently initiated in Manoa Valley, aimed at testing an ecological-evolutionary model of host-pathogen dynamics. The abundance and spatial distribution of host populations are considered in relation to infection prevalence rates estimated from PCR-based tests. The importance and potential value of host community interactions and extrinsic factors such as rainfall to an understanding of leptospirosis infection dynamics are highlighted.

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ROCKY MOUNTAIN SPOTTED FEVER IN THE UNITED STATES, 2003-2005

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Rocky Mountain spotted fever (RMSF) is the most commonly reported rickettsial disease in the United States. During 2003-2005, 4,755 cases of RMSF were identified via passive surveillance and reported via to the Centers for Disease Control and Prevention via the National Electronic Telecommunications System for Surveillance (NETSS); supplementary information reported via tick-borne rickettsial disease case report forms provided data for qualitative evaluation of RMSF risk factors, case severity, and diagnostic methodology. There were 1,091, 1,738, and 1,926 cases reported in 2003, 2004, 2005, respectively, continuing a trend of increased case reporting following 2000 (495 cases). There has been an almost a four-fold increase in the annual numbers of RMSF cases reported since 2000. Concomitantly, the annual RMSF incidence rate has increased more than 360% from 1.8 cases/million persons in 2000 to 3.8 cases/million in 2003, 5.9 cases/million in 2004, and 6.5 cases/million in 2005. Five states: NC (331, 535, 625 cases, respectively); OK (138, 190, 52 cases); TN (74, 117, 196); AR (48, 188, 137); and MO (51, 106, 140) accounted for 62% of cases reported during 2003-2005. CDC staff are investigating a number of reasons for these increases that include possible environmental and cultural factors (overall reductions in tilled land, increased intrusions into previously wild space with suburbanization, and increased outdoor activities) and factors that have influenced surveillance activities (heightened disease awareness and enhanced case-finding efforts) as well as changes in laboratory testing methods that may be affecting the numbers of presumed cases reported that are not adequately supported by clinical and laboratory evidence.

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DEVELOPMENT OF DRUG RESISTANCE IN *MYCOBACTERIUM TUBERCULOSIS*

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The control of communicable diseases is often complicated by the emergence of drug-resistant micro-organisms. In ***Mycobacterium tuberculosis*** (MTB), the causative agent of tuberculosis (TB), antibiotic resistance is predominantly established by point mutations in specific genes and on very rare occasions by insertion elements disrupting certain genes or by deletions causing frame-shifts. Uncomplicated cases of TB are typically treated with a combination of first-line drugs, containing at least isoniazid (INH) and rifampicin (RIF), the two most powerful and commonly used anti-TB drugs currently available. Multidrug-resistant TB (MDR-TB) is generally defined as being resistant to at least these two drugs, thereby clinically separating the uncomplicated TB-cases from those that are much more difficult to treat.

It is believed that in MDR-TB INH-resistance generally precedes resistance to RIF, based on the higher prevalence of clinical isolates of MTB with mono-resistance to isoniazid. However, we find that the spectrum of RIF-resistance conferring mutations emerging in some INH-resistant strains or strains resistant to other rifamycins diverges from the spectrum observed *in vivo*. In addition, certain RIF-resistance mutations are associated with moderate stress-responses, probably impacting upon the subsequent acquisition of drug resistance. Based on these results, we question the current dogma that the pathway from INH mono-resistance to RIF+INH-resistance is the most preferred route to MDR-TB. Furthermore, recent insights into the evolution of resistance suggest that the genetic routes to development of (multi)drug-resistance may be quite tightly constrained. These findings may have consequences on public health policies and treatment regimens.

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CHARACTERIZATION OF *STREPTOCOCCUS PNEUMONIAE* ISOLATES PREVALENT AT GA-RANKUWA HOSPITAL, SOUTH AFRICA

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Penicillin-resistant strains of S*treptococcus pneumoniae* are now found worldwide and strains with resistance to cephalosporin are being reported. Seventy four (74) strains of *Streptococcus pneumoniae* collected over a 13 month period (June 2003-July 2004) at the Ga-Rankuwa Hospital, South Africa, were characterized by determining their antibiotic susceptibility patterns, penicillin and cefotaxime MICs and serotyping using disc diffusion, E-test, and Quallung reaction, respectively. Polymerase chain reaction to identify penicillin resistance-associated genes- *pbp 1a, 2a,*

and *2x,* was also performed. Thirty two percent (24) of the isolates showed resistance to oxacillin, 12% (9) to clindamycin, 12% (9) to erythromycin, 12% (9) to tetracycline 5% (4) to cefotaxime, and 1% (1) to chloramphenicol. All isolates were susceptible to vancomycin. Twenty-five isolates (34%) were resistant to one or more antimicrobial agents. No high level resistance to penicillin G (> 2 µg/ml) was detected. Twenty different serotypes/serogroups (STGs) were represented in the total number of isolates and STGs 3 (n=12), 1 (n=11) and 6A (n=10) were the most common. Penicillin resistance occurred most commonly among the serotypes 14, 23F, 6A, and 6B. The isolate resistant to the most number of antibiotics had serotype 23F (resistant to all antimicrobial agents tested with the exception of vancomycin). Sixty- four percent of all isolates had one or more *pbp* genes. Penicillin- and cefotaxime- resistant isolates had *pbp 2x* and *1a* while *pbp2b* was seen on both resistant and susceptible strains. In conclusion, although 32 % of the isolates showed only intermediate resistance to penicillin, penicillin resistant genes were present in the majority (64%) of *Streptococcus pneumoniae* isolates at Ga-rankuwa hospital. The predominant serotypes were represented in the 23-valent vaccine.

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EFFICACY OF CARBEPENEMS IN THE TREATMENT OF A HAMSTER MODEL OF ACUTE LEPTOSPIROSIS

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Leptospirosis is an enzootic disease of global importance. It often presents as an undifferentiated fever, and can be difficult to distinguish from other febrile illnesses. In severe cases, empiric treatment often relies on broad spectrum antimicrobial use aimed at other disease processes such as bacterial sepsis. The carbepenems are broad spectrum antimicrobials which are often used as empiric treatment in such cases. These antimicrobials have ***in vitro*** activity against ***Leptospira***, but their efficacy ***in vivo*** is unknown.

Female Golden Syrian hamsters were inoculated intraperitoneally (IP) with 0.5 ml of Ellinghausen McCullough Johnson Harris medium containing 10^5 organisms of *Leptospira interrogans* serogroup Canicola serovar Portlandvere. Beginning on the second day after infection, groups of ten hamsters were treated daily for 5 days with IP doses of imipenem or ertapenem (5, 25, and 50 mg/kg). Untreated hamsters and hamsters treated with doxycycline (5 mg/kg IP daily) were used as controls. Hamsters were monitored 21 days for survival. All moribund animals were euthanized. All untreated hamsters died within nine days of infection. 95% (19/20) of control animals treated with doxycycline survived to 21 days. Treatment with 5 mg/kg of imipenem, 5 mg/kg of ertapenem, and 25 mg/kg of imipenem led to 90% survival. Animals treated with higher doses of imipenem and ertapenem developed diarrhea followed by dehydration and this led to a decreased survival rate in these groups compared to the lower doses. (50% survival with imipenem 50 mg/kg, 40% survival with ertapenem 25 mg/kg, and 0% survival with ertapenem 50 mg/kg). A representative stool sample from an animal displaying this toxicity tested positive for *Clostridium difficile* toxin. Though associated with toxicity at higher doses, the carbepenems were effective at preventing death from leptospirosis in this hamster model. Carbepenems are likely to be efficacious in humans with leptospirosis.

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COMPARISON OF DIFFERENT BLOOD CULTURE TECHNIQUES FOR THE ISOLATION OF *BRUCELLA* SPP. FROM PATIENTS IN PERU

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Brucellosis is a zoonotic disease caused by the transmission of *Brucella* spp. to humans through direct contact with infected animals or the consumption of non-pasteurized dairy products. The human pathogens *B. abortus, B. melitensis, B. suis* and *B. canis* have all been identified in South America. Isolation of the bacilli from blood is helpful for species determination and is used for diagnostic confirmation. An assay validation protocol was initiated to evaluate two culture methods, Ruiz Castañeda (RC) and lysis centrifugation (LC), of *Brucella* spp. from the blood of patients reporting to Daniel Carrion Hospital in Lima, Peru. A total of 38 Rose Bengal-positive samples from 25 acute, 6 sub-acute, 2 chronic and 5 relapse patients were collected over the course of 9 months and received at the Naval Medical Research CenterD Reference and Diagnostic laboratory for testing. A confirmatory sera agglutination antibody titer test was performed with a median titer of 1/400 (1/25 - 1/3000). Of the 38 blood samples cultured, 15 grew using the RC method (13 acute, 1 sub-acute and 1 chronic) and 20 grew using the LC technique (14 acute, 4 sub-acute, 1 chronic and 1 relapse). While the LC technique was not observed to be significantly more sensitive than the RC technique in culturing acute and chronic specimens, the LC method could provide an advantage in culturing sub-acute and relapse brucellosis samples. The major advantage in the LC technique, however, is the overall mean detection time observed (3.5 days) over the RC method (10.7 days, p=0.007). In addition, the difference between the mean detection times (RC-LC) was greater for those samples with serum agglutination titers 1/200 (10.3 days faster) than for those with titers >1/200 (7.7 days faster) thus strengthening the role of the LC technique for those samples with low confirming antibody titers. Another significant advantage of LC over the RC method is cost. The LC technique costs 40% less than the RC method in terms of material supplies and the shorter time to detection means significant savings in personnel cost and overhead, as well. In regions where resources are limited, the LC technique stands as a superior method. Our results confirm the utility of the LC technique for the culture of *Brucella* spp. from the blood of infected patients providing a cheaper, more rapid confirmatory diagnosis and appropriate antibiotic treatment.

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ANTIMICROBIAL PROPERTIES OF SELECTED HERBAL PREPARATIONS AGAINST STANDARD AND CLINICAL ISOLATES

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The study was to evaluate the antimicrobial activities of alcoholic extracts from eight [8] selected medicinal plants against pathogenic organisms isolated from clinical sources using agar diffusion method. The selected plants were *Xylopia aethiopica*, *Cinnamomum zeylanicum, Zanthoxylum xanthoxyloides[Fragara], Psidium guajava, Spondias mombin, Alcornea cordifolia, Tridax procumbens* and *Eugenia caryophyllata.* The pathogenic clinical isolates used were *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi,* Cand*ida albicans, Proteus mirabilis, Klebsiella pneumo pneumoniae.* Two different concentrations of Sixteen (16%) and thirty-two (32%) percent of the fractions each of the eight (8) medicinal plants were prepared. The antimicrobial effect of

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each concentration was measured by measuring the zones of inhibition in millimetres. Also measured were the minimum inhibitory concentrations (MICs) of the different fractions. The fractions produced significant zones of inhibition ranging from 6-25mm against the standard and clinical isolates. For instance, both the16% and 32% alcoholic fractions of *Eugenia caryophyllata* had 100% activity against the standard as well as the clinical isolates with zones of inhibition ranging from7-25mm. MIC was found to be 40mg/ml. The other seven fractions also inhibited all the standard and clinical isolates with the exception of *Klebsiella pneumo pneumoniae* with zones of inhibition ranging from 6-25mm and MICs ranging from 40-80mg/ml. The zones of inhibition form the 16% and 32%were found to be significantly not different. Thus, the fractions had antimicrobial properties and therefore could be developed to manage diseases caused by these pathogenic organisms.

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STUDY THE MAGNITUDE OF SEPTIC ARTHRITIS AMONG NEONATES ADDMITTED TO NICU

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Septic arthritis is rarely detected or may even pass unnoticed. The objective of this study was to answer if the neonatal septic arthritis is a common problem. The study included 395 cases with neonatal sepsis.Blood culture was done for all cases, and one case culture from joint aspiration, well as radiological studies. Septic arthritis was detected in 9 (2.27%) infant's. They all showed clinical, symptoms and signs of arthritis. Limitation of movement and pain with motion were seen among all cases i.e. in the nine cases (100%),then swelling of the joint which was detected in 8 cases (88.9%) followed by irritability among 7 cases (77.8 %). Single joint involvement was seen in 77.8% of cases.Hip joint was the commonest joint to be involved. The risk factors were; intravenous fluid in (100%), sampling femoral vein / artery for blood analysis (66.7%), prematurity, extravasations of fluid; cellulites and PRM were recorded in 5 cases (55.6%). We conclude that neonatal septic arthritis is not common feature of neonatal infection/sepsis. The most important leading criteria are parent's complaint; which is inability to move limb. Clinical evaluation is superior to laboratory findings in diagnosis of septic arthritis as laboratory findings are similar to all septic cases. Anticipation means early medication and avoidance of joint destruction. We recommend early diagnosis including frequent examinations of joints, prompt treatment and control of nosocomial infection are important in management.

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DEVELOPMENT A SUPERPARAMAGNETIC IMMUNOCHROMATOGRAPHIC TEST FOR CYSTICERCOSIS

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We have developed sensitive and specific cysticercosis and taeniosis ELISAs based on the CDC Western blots for cysticercosis and taeniosis. In many parts of the world, equipment to run ELISA or Western blot is not available. Also, in many cases a quick decision on the status of the patients is needed. Immunochromatographic test (ICT), commonly referred to as dipstick or lateral flow assays, is a rapid test, but may lack sensitivity. A new development for ICT is the use of superparamagnetic particles as the reporter of the antibody-antigen reaction instead of gold particles. The magnetic signals then are detected by a magnetic reader (a hand-held or a bench one). This format, in our preliminary study, has sensitivity comparable to our ELISA. Another advantage of this format is that it allows us to combine tests for cysticercosis and taeniosis in one strip and enables us to diagnosis two conditions at once. Development of this test will allow us to detect diseases in the field rapidly with sensitivity comparable to ELISA.

(ACMCIP Abstract)

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STUDY OF PIG CYSTICERCOSIS IN AN INDUSTRIALIZED FARM USING ELECTROIMMUNOTRANSFER BLOT (EITB)

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Taenia solium Cysticercosis is an important parasitic disease that affects both humans and pigs in developing countries. Recently, the numbers of human cysticercosis cases in industrialized countries appears to be increasing proportional to immigrants from endemic areas. The parasite life cycle includes humans as definitive host, and pigs as intermediate host. Although pigs are the normal intermediate hosts, humans can also develop the larvae stage after accidental ingestion of tapeworm eggs. Cysticercosis is associated with poor sanitary conditions and inadequate hygiene practices in small farms. In Peru, these characteristics can be identified in rural areas where prevalence and incidence have been reported. For this reason; it is unlikely to expect the presence of the disease in farms with high levels of bio-security. Until now, there was no report on the cysticercosis situation in technologically advanced farms in Peru. Therefore, the aim of this study was to evaluate the prevalence and incidence of cysticercosis in pigs in a technologically advanced farm in Lima using EITB-C. The results of our study showed high seroprevalence (22%, 116/528). Moreover, on a second sampling performed one year later the seroprevalence increased by two-folds (46%, 240/521) leading to an accumulated incidence of 50% (94/187). After evaluating the preliminary results, we met with farm workers to explain the disease transmission, and pigs were treated once with oxfendazole. These results reveal a seroprevalence and an incidence rate as equal or more as in the Peruvian rural zones where the disease is endemic. This suggests that pigs might be constantly exposed to environmental *T. solium* eggs-contaminated conditions.

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DIFFERENTIATING *TAENIA SP* EGGS- DOES ZIEHL NEELSEN STAINING HELP?

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Three big tapeworms lodge in the human intestine: *Diphyllobothrium sp*, *Taenia solium* and *Taenia saginata*. Differential diagnosis is based on morphology of the adult tapeworm scolex or proglottids, not always available. While *Diphyllobothrium* eggs are easily distinguishable, eggs from *T. solium* and *T. saginata* can not be distinguished by microscopy. Old literature reports mentioned the use of the Ziehl Neelsen staining to distinguish *Taenia* eggs. We stained pre-existing archive parasite material (gravid proglottids obtained from 19 *T. solium* and 8 *T. saginata* carriers), archive stool samples 12 from *T. solium* and 11 *T. saginata* carriers, and freshly collected pre-treatment samples from 6 *T. solium* and 2 *T. saginata*

carriers. In *T. solium*, as the embryophore matures and becomes thicker, coloration gets more intense, departing from blue to gradually acquire the magenta tones. *T. saginata* samples from distal gravid proglottids have no blue areas. Conversely, *T. solium* eggs in distal proglottids showed a mixture of blue and magenta tones. There was no difference in staining of eggs from fresh versus preserved stool samples. While Ziehl Neelsen staining distinguished fully mature *T. solium* from *T. saginata* eggs in most cases, this distinction is by no means absolute and carriers some degree of subjectivity. The data suggests different expression biochemical processes in egg maturation between these two species. Given that T. *solium* infect human host and cause cysticercosis, unlike *T. saginata*, further understanding and characterization of these differences may provide diagnostic, treatment, and potential vaccine targets.

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RESIDUAL BRAIN CALCIFICATIONS IN NEUROCYSTICERCOSIS

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A 33 years old female Peruvian patient presented with a 10-years history of secondary generalized seizures. Initial neuroimages showed brain lesions compatible with cysticercosis including an extensive subarachnoid lesion in the right temporo-parietal convexity and multiple intraparenchymal viable cysts and calcifications. She received an initial course of albendazole at 15 mg/kg/d, stopped at day 15 because of increased liver enzymes. The subarachnoid lesion markedly decreased in size and most parenchymal cysts resolved. After one year a ventricle-peritoneal shunt was placed and a second course of albendazole was needed because of re-growth of the subarachnoid lesion and persistence of four viable parenchymal cysts. All cysts resolved after a second course of anti-parasitic treatment. Neurocysticercosis is a frequent cause of seizures in most of the world. This case illustrates the effect of anti-parasitic therapy to kill parasite cysts, the frequent need for successive courses of therapy, and the potential of subarachnoid lesions to grow and cause obstructive hydrocephalus. Even after successful anti-parasitic treatment, the many remaining calcified scars persist, acting as potential foci of relapsing inflammation and seizures.

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DEVELOPMENT OF AN AUTOMATED COPROANTIGEN ASSAY USING THE TRITURUS ANALYZER

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The parasite *Taenia solium* can cause two diseases in humans, taeniasis and cysticercosis. Neurocysticercosis, when larval cysts invade the central nervous system has been declared "the most important neurological disease of parasitic origin in humans" by the World Health Organization. Diagnosis and treatment of taeniasis are essential for the control and elimination of cystcercosis. We describe the optimization of reagents for an ELISA coproantigen assay to detect *T. solium* antigens in stool samples using the automated Triturus autoanalyzer (Diagnostic Grifols, Spain). After optimization of this assay, stool samples from *T. solium* tapeworm carriers, proven negative samples and potential cross reactors were tested. A total of 926 stool samples were tested, 212 from parasitologically confirmed tapeworm carriers and 714 stool samples from residents of non-endemic areas, who were healthy or infected with intestinal pathogens other than *T. solium*. The sensitivity of the assay was 94.8% and specificity of 98.2%. These data suggest that the coproantigen assay run on the Triturus analyzer is a reliable assay and useful when testing large number of samples. The assay is being evaluated for development of a commercial kit so the test will be readily available, standardized and less susceptible to human error.

(ACMCIP Abstract)

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THE EFFECT OF TIMING AND FREQUENCY OF MALARIA INFECTION DURING PREGNANCY ON LOW BIRTH WEIGHT AND MATERNAL ANEMIA

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Background: In areas of endemic transmission, malaria in pregnancy is associated with severe maternal anemia and low-birth weight (LBW) deliveries. However the effect of timing and frequency of malaria infection on the severity of these adverse effects is not known. We conducted a prospective observational study recruiting 2,462 pregnant women in Blantyre, Malawi. The presence of malaria was assessed by microscopy at scheduled visits during follow-up and delivery. Birth weight and maternal hemoglobin levels were measured at delivery. Women were given sulfadoxine-pyrimethamine during follow-up as presumptive therapy. The prevalence of LBW and anemia at delivery in relation to the frequency and timing of malaria infection was analyzed using a binomial regression model. Compared with women who had no malaria during pregnancy, the prevalence of LBW increased with the number of malaria episodes; 1 episode of malaria (prevalence ratio [PR] =1.52; 95% C.I. 1.06-2.18) and 2 episodes (PR= 1.98; 95% C.I. 1.20-3.26). The prevalence of anemia also increased with the number of episodes of malaria: 1 episode of malaria (PR= 1.15; 95% C.I. 0.89-1.45) and 2 episodes of malaria (PR= 1.90; 95% C.I. 1.39-2.59). Timing of malaria infection had an effect on prevalence of LBW. Compared with women who had no infection, the prevalence of LBW was highest in women with infection in both antenatal period and delivery (PR=2.21; 95% C.I. 1.34-3.65), then antenatal period only (PR= 1.55; 95% C.I. 1.01-2.37), and at delivery only (PR =1.48; 95% C.I. 0.93-2.34). The prevalence of anemia was also higher in women who had malaria both in the antenatal period and delivery (PR= 2.01; 95% C.I.1.45-2.76), then at delivery only (PR=1.31; 95% C.I. 0.90-1.90), and in the antenatal period only (PR=1.11; 95% C.I. 0.83-1.48). In conclusion, iming and frequency of malaria infection during pregnancy affect the severity of malaria complications during pregnancy.

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ASSESSMENT OF PHARMACOCEUTICAL CAPABILITY OF INDIGENOUS PLANT EXTRACTS IN VISCERAL LEISHMANIASIS

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The Anti-***Leishmania donovani*** effect of 16 medicinally important indigenous plant extracts have been studied in differential dose concentration (0.01mg, 0.1mg or 1mg per ml culture) and microscopically

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tested at 24h and 48h of incubation with 2x10^6 ***L. donovani*** promastigotes. The results showed that the plant extracts of ***Azadirachta indica, Eclipta alba*** and Agave americana potentially inhibited ***Leishmania*** growth in 100% when compared to control (1 µg/ml Amphotericin-B or 20 µg/ml PHA treated culture). By contrast ***L. donovani*** when stimulated with SAG continued its replication as SAG could regulate only 63% ***Leishmania*** inhibition after 24h. Plant extracts identified with anti-***Leishmania*** effect were further tested for their effect on replication of amastigotes. Briefly ***L. donovani*** promastigotes initially grown at 24°C were further incubated at 37°C in RPMI-1640 with 20% FCS for one hour and continued for another one hour in presence of plant extracts and Amphoterisin-B in individual batches. Cells harvested after one hour were stained with propidium-iodide and the percentage of dead cells were studied using cell-quest software on FACS-Calibur. It was shown that although all plant extracts excelled through anti-***Leishmania*** activity, but plant extract of ***A. americana*** in particular made more impact as about 90% axanic amastigote inhibition was seen with ***A. americana*** treated culture, compared to 73.52% inhibition or shown by Amphotericin-B. The results obtained in the present study are significant in light of new drug development for the control of Visceral Leishmaniasis.

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MUCOSAL LEISHMANIASIS IN A CENTRAL AMERICAN IMMIGRANT DIAGNOSED WITH REAL-TIME PCR: CASE REPORT AND REVIEW OF DIAGNOSTIC AND TREATMENT ISSUES

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Mucosal leishmaniasis (ML) is rarely reported in the U.S. despite increasing immigration from endemic countries. We report the case of a Honduran immigrant residing in the Washington DC area for 13 years prior to undergoing resection of a longstanding nasal polyp. A postoperative "cellulitis" ensued, progressing through multiple courses of antibacterial treatment over a six month period. A Taqman™ based real-time PCR assay for Leishmania genus specific 16s ribosomal RNA gene developed at our institution provided a putative diagnosis of ML within 48 hours of biopsy. Subsequent examination of leishmania culture demonstrated the presence of mobile flagellates and review of previously obtained histopathologic specimens revealed a lone amastigote, thus securing the diagnosis of ML. The patient recalled a childhood history of ulcerating skin lesions while residing in Honduras. He completed a 20 day course of amphotericin B deoxycholate without complication and achieved near complete healing of the cosmetically debilitating lesion. Diagnostic issues pertaining to the difficulties of diagnosing ML are addressed with specific attention to the role of PCR as an adjunct to traditional methods. Literature is also reviewed focusing on contrasting treatment options for ML juxtaposed with that for other forms of Leishmaniasis. Conclusions: 1. ML should be suspected even in immigrants long departed from endemic areas; 2. The incidence of ML in this country is likely to increase; 3. Real time and conventional PCR are useful, timely adjuncts to traditional diagnostic measures.

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DHEAS PREDICTS IMPROVED NUTRITIONAL STATUS IN HELMINTH INFECTED CHILDREN, ADOLESCENTS AND YOUNG ADULTS IN LEYTE, THE PHILIPPINES

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Pubertal development and associated downmodulation of proinflammatory cytokines may predict improved nutritional status independent of chronic parasite infections in developing countries. We enrolled 731 individuals aged 7-30 years from Leyte, the Philippines, where helminth infections and nutritional morbidity are highly prevalent. The following data were collected: venous blood for hemoglobin, ferritin, dehydroepiandrosterone sulfate (DHEAS), C-reactive protein (CRP) and serum proinflammatory cytokines (interleukin [IL]-1, IL-6, tumor necrosis factor [TNF]-α, and soluble TNF receptor I [sTNF-RI]); anthropometric measurements to calculate upper arm muscle area Z-score (UMAZ) and sum of triceps and subscapular skinfolds Z-score (SSFZ); stool to determine Schistosoma japonicum and geohelminth egg counts; and questionnaires assessing socio-economic status (SES). In cross-sectional multilevel linear and logistic regression analyses, adjusted for confounders, relationships were assessed between 1) DHEAS and nutritional status, 2) DHEAS and proinflammatory cytokines, and 3) nutritional status and proinflammatory cytokines. Independent of age, SES and helminth infections, increased levels of DHEAS were associated with improved nutritional status and decreased prevalence of non-iron deficiency anemia in both males and females. DHEAS showed a dose-dependent inverse relationship with CRP and production of IL-6 (P=0.08 and <0.0001, respectively). These inflammatory markers, in turn, were consistently associated with nutritional morbidity. These results suggest that the puberty-associated rise in DHEAS downmodulates proinflammatory immune responses and thereby reduces nutritional morbidity in a population experiencing a high burden of chronic helminth infections. This novel regulatory mechanism of inflammation-related nutritional morbidity emphasizes the importance of treating pre-pubescent children for helminth infections.

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POPULATION PHARMACOKINETIC MODELLING OF COMMON ANTIMALARIAL DRUGS IN MALAWI

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Drug resistant falciparum malaria is a major public health problem throughout Africa. Much of the failure of antimalarial therapy is due to resistant parasites, but some failures may have a pharmacokinetic (PK) basis. This study aimed to develop population PK models for sulfadoxine (SDX), pyrimethamine (PYM), chloroquine (CQ), amodiaquine (AQ) and its metabolite des-ethyl-amodiaquine (AQm) in young Malawian children with uncomplicated falciparum malaria. The work was nested within a randomised controlled trial (RCT) comparing SP alone with SP + 3 days of CQ, AQ or artesunate (ART). The dose of SP was 25mg/kg SDX and

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12.5mg/kg PYM, CQ was 25mg/kg/day, AQ was 30mg/kg/day and ART was 4mg/kg/day. 455 children aged >1year and < 5years were recruited, and an additional 35 children were recruited to the study unblinded for more intensive blood sampling to provide richer PK data. HPLC methods were developed in Malawi for SDX, PYM, CQ, AQ and AQm from whole blood. Concentration vs. time profiles were established for SDX (200 children) PYM (180), CQ (100) and AQ/AQm (80). Population PK models are being developed from these data and will be presented. The models will be used to examine individuals in the RCT to see whether treatment failures can be explained by inadequate drug exposure. The models will also be used (a) to look for co-variants influencing drug PK parameters within an individual and within the population, and (b) to simulate different dosing regimes to optimise drug exposure.

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ASSESSMENT OF THREE NEW PARASITE LACTATE DEHYDROGENASE (PAN-PLDH) TESTS FOR DIAGNOSIS OF UNCOMPLICATED MALARIA

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Rapid Diagnostic Tests (RDTs) represent an alternative to microscopy for malaria diagnosis. RDTs which detect Histidine Rich Protein 2 (HRP2) only detect falciparum malaria and remain positive for several weeks after treatment. A second generation of tests which detect parasite lactate dehydrogenase (pLDH), has the advantage of detecting all 4 plasmodium species, and of returning to negative more rapidly after treatment. There is need to evaluate the new pLDH tests in field conditions before their wider use. The objective of this study was to assess the performance of three new pLDH tests, namely Vista-pan malaria test® (Mitra, India), CareStart antigen test® (AccessBio, USA) and Parabank device® (Orchid/Zephyr, India). Results were compared to that of an HRP2 test, Paracheck-f® (Orchid/Zephyr, India). Patients with suspected malaria were screened at Mbarara hospital, Uganda. Each RDT was read blindly by two independent readers and results were compared to microscopy. Outcomes were: validity (sensitivity, specificity, positive and negative predictive values); inter-reader reliability (kappa coefficient); percentage of positive tests during a two week post-treatment (Coartem®) follow-up; ease of use as assessed by the technicians who completed a questionnaire at the end of the study. 248 blood smear positive and 212 negative patients were recruited. Sensitivities above 90% were shown by 2 pLDH tests [Carestart (95.6%), Vistapan (91.9%)] and by Paracheck (94%), whereas Parabank was less sensitive (84.7%). Specificities above 90% were shown by two pLDH tests [Parabank (94.6%), Carestart (91.5%)], whereas VistaPan and Paracheck were less specific (89.6% and 87.3% respectively). Sensitivity decreased with lower parasitaemias (Chi2 trend p<0.0001), however all tests achieved sensitivity >90% at parasitaemias ≥100/µl. All tests were highly reliable (Kappa>0.95). The percentage of positive tests after treatment was significantly higher for Paracheck: 69.7% at day 14, vs. 9.5% (Carestart), 8.9% (Vistapan), and 4.6% (Parabank). All tests had similar ease of use. In conclusion, the Carestart was considered the best of the three pLDH tests evaluated, although Vistapan was also considered a good alternative. Currently, the cost of these pLDH tests vary from USD 0.60 to USD 1.00 per test, as compared to Paracheck (USD 0.45/test). The higher cost of these tests needs to be addressed.

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SURVEILLANCE OF CHIKUNGUNYA VIRUS INFECTIONS IN JAKARTA

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The first recorded epidemic of Chikungunya virus (CHIK) was reported in the southern region of the Indonesian island of Sumatra in 1982. Since then, outbreaks of Chikungunya have occurred in cities/districts throughout Indonesia. From 2001 to March 2004, 28 serologically defined outbreaks of CHIK were identified predominantly in rural areas. However, the prevalence of CHIK and its potential spread in a sprawling urban center such as the capital city of Jakarta remains unknown. The potential for large outbreaks in Jakarta would likely be enhanced by the high indexes of the primary vector of CHIK, the *Aedes aegypti* mosquito. Here we report findings from surveillance for CHIK infections conducted at five primary health centers in Jakarta from May 2005-May 2006. A total of 290 participants were enrolled and 411 sera tested by RT-PCR and ELISA. Baseline serology for CHIK was identified among populations in all sub-districts ranging from 5.3% to 12.1%. Furthermore, recent infection was documented in 41 patients residing in South Jakarta. Fever, arthralgia, headache, malaise, myalgia and joint swelling were the prominent clinical manifestations associated with CHIK infection. These results reveal that CHIK virus has likely circulated in Jakarta for some time, and new outbreaks occur sporadically.

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EVALUATION OF MULTI-DIP-S-TICKS SDLST IN AN ENDEMIC POPULATION ON THE THAI-MYANMAR BORDER

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An evaluation of Multi-Dip-S-Ticks SDLST, a rapid dipstick diagnostic test for salmonella, dengue, leptospirosis, scrub typhus, and murine typhus (Multi-Test, PanBio INDX, Inc.), was performed as part of a fever surveillance study on the Thai-Myanmar border. Reference diagnostic tests and Multi-Tests were performed on acute and convalescent sera for leptospirosis, scrub typhus, and dengue fever and results were compared at varying Multi-Test dot intensity cutoff scores. The reference test for leptospirosis was a commercial enzyme-linked immunosorbent assay (ELISA) combined with confirmatory microscopic agglutination testing (MAT). For scrub typhus and dengue fever the reference tests were in-house ELISAs. Sensitivity, specificity, and overall agreement of the Multi-Test compared to reference diagnostics were low overall. For leptospirosis, sensitivity was 58% and specificity was 77% for convalescent specimens at a dot intensity cutoff of 1+. For scrub typhus, sensitivity was 26% and specificity was 95% for convalescent specimens at a cutoff of 1, and for dengue fever sensitivity was 78% and specificity was 36% for convalescent specimens at a cutoff of 2. Sensitivity was lower for acute specimens. Overall agreement at these dot intensity cutoffs was 74% for leptospirosis, 86% for scrub typhus, and 37% for dengue fever. In addition, sensitivity appeared to be low for five likely cases of typhoid fever identified by PCR and clinical criteria. Multi-Test does not appear to reliably differentiate illness caused by these four pathogens in this endemic population.

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RANDOMISED CONTROLLED TRIAL OF INTERMITTENT PREVENTIVE TREATMENT IN SCHOOLCHILDREN: IMPACT ON MALARIA, ANAEMIA AND SCHOOL PERFORMANCE

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Although malaria risk is greatest in early childhood, significant numbers of school-aged children remain at risk from malaria morbidity. Asymptomatic infection also contributes to anaemia, reducing concentration and learning in the classroom. An effective intervention in infants and pregnant women, intermittent preventive treatment (IPT) could be a valuable addition to school health programmes. A randomised controlled trial in Western Kenya was undertaken to examine whether IPT in schools can reduce parasitaemia and anaemia amongst schoolchildren and improve school performance. A cluster-randomised placebo-controlled trial was carried out during 2005-2006 among schoolchildren living in Bondo District, W Kenya: an area of intense perennial transmission. Thirty primary schools were randomly selected to take part in the trial and randomly allocated to intervention or control arms. Children with individual informed parental consent were eligible for treatment (age range 4-16y). IPT was administered 3 times per year, given once per term, in intervention schools. Mass treatment with anthelminthics was given in all schools. Pre- and post-intervention surveys were carried to establish haemoglobin level, prevalence and intensity of *P. falciparum* and intestinal helminth infections, and nutritional status. Class-based attention tests were conducted amongst children aged 10-14y on the day prior to clinical assessment. During the intervention period, children in stds 6 and 7 were taught a common curriculum in social studies in all study schools and later tested to compare learning in the intervention and control arms. The results of the trial (ended April 2006) will be presented, including impact on prevalence and intensity of *Plasmodium falciparum* infection; mean haemoglobin level and prevalence of anaemia; as well as impact on attention in class and learning.

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CLINICAL AND LABORATORY FEATURES OF CONGENITAL MALARIA IN NIGERIA

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Congenital malaria was thought to be rare, therefore screening for malaria parasite in neonatal infections had not been routine even in malaria-endemic regions. The clinical distinction between neonatal sepsis and congenital malaria has also posed a challenge. With increasing isolated reports of neonatal malaria in the region, a national multi- center study was carried out in Nigeria, West Africa, over a period of 12 calendar months to investigate patent malaria parasitaemia in the first 4 hours of postnatal life. Clinical and laboratory features of neonates found with malaria parasitaemia are described in this report. Mothers delivering in secondary and tertiary health institutions in four geographical zones of Nigeria from April 2003 - March 2004 who had live births, continuous residence within area of study center >2 years till time of study and provided informed consent were recruited. At delivery, thick and thin blood smears were made from blood taken from the mother (finger prick), placental aspiration, cord blood and from the baby (heel prick). Using standard techniques for Giemsa staining and light microscopy, malaria diagnosis was made on identification of asexual stages of Plasmodium. Patent *P. falciparum* parasitaemia was found in 5.1% of the neonates, all of who were full terms. 38.9% (37/95) of them had symptoms, the most frequent being fever in the first 4 hours of life (100%) and refusal to suck (10%). Their mean haematocrit was 48.27 ± 5.78% and mean parasite density was low. Concordance was good across sites between the occurrence of maternal, placental, cord and neonatal parasitaemia, (χ^2 =484.55, OR= 64.75, RR= 9.72, ρ=0.000 ~ P<0.0001. Antepartum maternal and placental parasitaemia were the most important risk factors for patent neonatal parasitaemia (χ^2=13.96, p=0.001 OR=4.3 and χ^2=457.74, p=0.000 [p<0.0001] OR=4.9) and more so in the symptomatic babies. Ten percent of their mothers were HIV-infected. In malaria-endemic countries, there is a need to evaluate the sick newborn for malaria and increase use of malaria prophylaxis in pregnancy.

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NOTIFICATION PATTERNS OF ACUTE RESPIRATORY INFECTIONS BASED ON LOCAL CLIMATE IN PERU

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Local climate appears to affect the epidemiology of respiratory tract infections (ARI), modifying their distribution during the year. Peru has a diversity of climate patterns that allow comparison of notification rates of ARI within one reporting system, including tropical rainforest, coastal desert (subtropical), and southern highlands (temperate). This study was undrtaken to evaluate the notification distribution patterns of ARI in the population of children under five years old over seven different geographic regions of Peru. We analyzed the ARI data collected by the Peruvian National Surveillance System from 2003 to 2005. We stratified the data in seven well-defined geographic regions: North, central and south coast; north, central and south highland; and rainforest. We determined the notification rates (NR) for upper respiratory infections, non-complicated pneumonia and complicated pneumonia for each region and for each year. Finally, we compared the curves of distribution among these regions. The coastal regions have the highest NR for upper respiratory infections: 192,337 per 100,000 for south coast; 131,101 per 100,000 for central coast and 129,095 per 100,000 for north coast. The rainforest (1,709 per 100,000), south coast (1,388 per 100,000) and south highland (1,204 per 100,000) regions present the highest NR for non-complicated pneumonia. On the other hand, the south coast (799 per 100,000) and south highland (715 per 100,000) regions show the highest NR for complicated pneumonia. Only the south regions (non-tropical coast and highlands) showed a perfect seasonal pattern with a peak during the Southern Hemisphere winter. The other regions have a stable pattern during the whole year. In conclusion, two patterns for ARI were observed, and these correlated with climate. The southern, temperate regions demonstrated a classical once a year peak in incidence whereas the sub-tropical and tropical regions demonstrated stable transmission throughout the year. Higher ARI rates in the coastal regions are probably influenced by a better access to health facilities. However, complicated pneumonia NR was higher in the south.

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HIGH MALARIA MORTALITY DURING MALARIA EPIDEMICS IN KENYA, BURUNDI AND ETHIOPIA

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Plasmodium falciparum (Pf) malaria epidemics are poorly documented. Field-based evidence to document their impact on mortality is largely missing. Current mortality estimates rely on extrapolations of limited site-specific or empirical observations. We report community-based mortality findings from three Pf malaria epidemics in Kenya, Burundi and Ethiopia. We performed retrospective mortality surveys in five sites during three malaria epidemics in Wajir District (Kenya (1998), Kayanza, Karuzi and Ngozi Provinces (Burundi, 2000 - 2001) and Damot Gale District (Ethiopia, 2003 - 2004). We selected subjects using two or three-stage cluster sampling. We calculated crude (CMR) and under five (U5MR) mortality rates, expressed as deaths per 10 000 persons per day. Mortality rates were compared to standard emergency thresholds of 1 death/10,000/day (CMR) and 2 deaths/10,000/day (U5MR). Using a clinical case definition, we calculated percentage of malarial deaths. Excess mortality due to malaria was estimated by applying the specific mortality rates due to self-reported malaria to the population and time period covered by the survey. In three sites, CMR were 2 to 9 times above the standard emergency threshold. In all sites, U5MR were 1.5 to 13.5 above the emergency threshold. Malarial deaths represented 51.7%-78.3% of all deaths, and 53.0%-82.3% of deaths in children under five. We estimate that between 12 223 and 26 612 deaths in the total population and between 4867 and 14 256 deaths in children under five were probably due to malaria. In conclusion, malaria epidemics are responsible for a high number of deaths and should be considered medical emergencies Earlier detection and better case-management are needed to decrease the high public health burden of malaria epidemics.

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THE IMPACT OF ANAEMIA, FALCIPARUM MALARIA AND MALNUTRITION ON THE PSYCHOMOTOR DEVELOPMENT OF YOUNG CHILDREN EXPOSED TO MALARIA TRANSMISSION

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The objective of this study was to determine the effects of malaria, anaemia and malnutrition in infants, in an area of perennial and intense malaria transmission. 661 Tanzanian children at 2, 8, 12 and 18 months of age were examined. The study was carried out within an intervention trial of malaria chemoprophylaxis and/or iron supplementation for the prevention of malaria and anemia. Children found to be severely anemic were withdrawn from receiving study interventions. The psychomotor development was assessed using a developmental test adapted from the Bayley's scale of infant development. Four different areas were evaluated: behaviour, hearing and speech, gross motor, and hand manipulation. Muscular tone and the state of consciousness were also examined. Non-complicated malaria episodes were associated with a higher risk of abnormality in the state of awareness (OR 2.88; p=0.026) at 8 months of age and of behaviour (OR 1.75; p=0.036) at 12 months of age and in the hearing and speech area (OR 2.12; p=0.051) at 18 months of age. An episode of anemia was associated with higher risk of abnormality in the gross motor area at 12 and 18 months respectively (OR 5.48; p=0.003; OR 1.19; p=0.038). Malnutrition was associated with a consistent increased in the abnormality of the gross motor area at 2, 8 and 12 months of age, and with a negative effect on the behavioural, hand manipulation and hearing and speech areas at 12 and 18 months. In conclusion, malaria and anemia episodes during the first year of life may affect negatively the psychomotor development of infants. Malnourished children had a poor performance of the gross motor and of the hearing and speech area. Whether these effects are sustained needs to be evaluated in longer prospective studies. Effective control measures that reduce malaria, anemia and malnutrition are likely to have an impact on these outcomes and should start early in life.

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DEVELOPMENT OF HOME BASED MANAGEMENT OF ADENOLYMPHANGIADENIATIS AND LYMPHOEDEMA IN A FILARIA-ENDEMIC AREA OF NORTH EASTERN NIGERIA

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A recent comparison of the health facility-based management approach with other strategies for the management of lymphoedema showed that home based approach is more acceptable to patients in Nigeria. However, in order to understand benefits of the home based management approach a demonstration was carried out in three endemic local government areas in North-eastern Nigeria. Information, education and communication (IEC) materials on management of lymphoedema and ADL were designed, developed, tested and adapted for local use. Advocacy and mass mobilisation for recruitment of patients, traditional healers, school hygiene teachers and community based organisations into the demonstration programme was also carried out. Patients, their families and other stakeholders were trained to manage ADL and lymphoedema under the broad supervision of the health system. A network of NGOs with interest in control of lymphatic filariasis was formed and responsibilities agreed upon. Advocacy was also promoted through a nationwide prime time television news item which focused on the problem, in addition to coverage by five national newspapers. More than 480 patients were recruited and trained for the demonstration. The patients established 35 support groups in which about 75% of the patients were active members. The support groups provided psychosocial support and encouraged treatment compliance through peer group education and motivation. Educational card and board games were distributed to each support group and used for promotion of appropriate health practices within the groups. 187 health personnel at the community level were trained in addition to 56 health supervisors to provide first line support to the patient groups. 48 school hygiene teachers played a significant role in supervising the support groups and in reducing stigma and changing behaviour towards the victims of this disease in the communities. 26 traditional healers were also trained and 84.6% of them have discarded the use of incisions and have become allies in appropriate care-giving. The demonstration raised awareness nationwide and a flood of inquiries from patients in different parts of the country indicates that the problem not only much more widespread than assumed but there is great concern among patients to find relief.

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PREVIOUS EXPOSURE TO ANTIBIOTICS INCLUDING ANTITUBERCULOTIC AND ANTIFUNGAL AGENTS WITH AND WITHOUT PROPHYLACTIC TMP/SMX AND RESISTANCE IN BACTERIA FROM HIV POSITIVE CHILDREN: ABSENCE OF RESISTANCE SELECTION PRESSURE OF TMP/SMX

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Most of HIV positive children receive TMP/SMX prophylaxis, repeated courses of treatment for candidiasis and several antibiotics due to repeated

respiratory tract infections. About one third of all children with HIV/AIDS in developing countries are concomitantly treated for tuberculosis.

The aim of this research was to assess the role of TMP/SMX and therapeutic administered antibiotics or antifungals in selecting resistant bacterial mutants in children with HIV/AIDS in Phnom Penh,Cambodia.

We compared 5 groups of children and their colonization with resistant phenotypes of bacteria:not receiving any antibiotic, antifungal or antituberculotic agents 1 month before positive culture for resistant phenotype (A), receiving TMP/SMX only (B), receiving TMP/SMX with any antibiotic, antituberculotic or antifungal agents (C) and receiving any antibiotic, antifungal or antituberculotic agents without TMP/SMX (D). Children not receiving any antibiotic had similar occurrence of phenotypes of multiresistant bacteria or Candida spp. than those receiving TMP/SMP prophylaxis (B) or receiving only ATB treatment plus TMP/SMX prophylaxis (C) or ATB/antiTB treatment alone (D). Of 62 children colonized and exposed to TMP/SMX and other antibiotic, antifungal or antituberculotic agents, 36% isolates were MRSA, 27% Candida spp., 15% were ESBL producing Enterobacteriaceae and 14% penicillin resistant pneumococci.

Assessing prior exposure with one antibiotic only, there was no major difference of a particular pathogen relationship to any particular antibiotic. When comparing children with prophylactic antibiotic therapy with or without TMP/SMX, those children colonized with fluconazol resistant Candida spp. had significantly higher prior exposure to fluconazol with or without TMP/SMX (P<0,0004). Candida albicans was also significantly associated with prior exposure to ciprofloxacin (P<0,0004) with or without TMP/SMX.

When comparing children after TMP/SMX prophylaxis plus other antibiotic therapy, 3 significant associations appeared: MRSA and AMO+TMP/SMX exposure (P<0,04), Candida albicans and CIP+TMP/SMX exposure (P<0,001), NAC FLU-R and fluconazol exposure (P<0,0006). Comparing previous exposure of ATB plus TMP/SMX versus exposure of TMP/SMX only, no differences between both groups have been observed - TMP/SMX does not contribute to less or more resistance in MRSA, PRP, ESBL producing Enterobacteriaceae.

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EFFECTIVENESS OF ARTEMETHER -LUMEFANTRINE IN UNSUPERVISED HOME TREATMENT OF UNCOMPLICATED MALARIA

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In line with WHO recommendations, Nigeria is committed to introducing artemether-lumefantrine (Coartem®) an ACT at both health facility and community level through the home management of malaria (HMM) strategy. No study has been reported on the use in HMM context in Nigeria. Hence, this study sought to establish the effectiveness of Coartem® in home/community treatment of presumed malaria.

Community based drug distributors (CBDs) in 7 villages in Ona-Ara Local Government, Oyo State, Nigeria were trained on signs and symptoms of malaria, use of Coartem®, and preparation of thick blood film. The CBDs in turn trained their community members on these topics. Treatment guidelines were distributed to CBDs and households in the communities. Follow-up study of children aged 4 - 59 months presenting with fever to CBDs began in October, 2005 and still on-going. Thick blood films for microscopy and Whatman filter paper for PCR analysis were prepared by CBDs from each patient at presentation (D0) and on Day 28 (D28) for those parasitaemic on D0. Laboratory assistants collected slides daily for screening and results were provided to the CBDs within 24 hours of collection. Caregivers were interviewed on D28 on how drug was administered and occurrence of adverse event. Two hundred and eighty seven patients have been enrolled to date and 209 (72.8%) had parasitaemia at DO. 131 of the 209 (62.7%) patients reported for D28 follow-up and 54 of them had patent parasitaemia - D28 cure rate of 58.8%. The PCR analysis is yet to be completed. Eighty-two of 131 (62.6%) mothers who reported on D28 were interviewed. Seventy four (90.2%) of them reported they used Coartem® correctly and only 9 (11.0%) reported adverse event in their children. All the mothers found the guidelines useful and were of the opinion that the drug was effective. A failure rate of 41.2% is above figures reported in clinical trials using Coartem® in Nigeria and Africa and is a source of concern for a drug that is yet to be introduced for widespread use in the community. However, some of the cases with parasitaemia at D28 may be re-infections especially as a high drug dosage compliance rate was recorded by mothers. PCR analysis of D28 samples would be valuable in distinguishing re-crudescent infections from re-infections especially in this area of intense malaria transmission. Provision of adequate IEC materials stands to increase compliance to correct use of

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THE BURDEN OF MALARIA AND ITS MANAGEMENT IN ONCHOCERCIASIS ENDEMIC RURAL COMMUNITIES OF IMO STATE, NIGERIA

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The present study was conducted on 350 women to assess the added burden of malaria on women suffering from onchocerciasis in the Ezenachi communities of Okigwe L.G.A.of Imo State Nigeria. Thick and thin blood smear method was used for parasitemia.The prevalence of malaria was 41 % (27.6% in females and 13.4% in males) which is an added stress on these women. These women constitute the major work force and when affected with onchocerciasis suffers debilitation as well as blindness as the disease manifest itself with various skin, ocular, lymphatic and systemic signs. The pattern of socio-economic liability due to onchocerciasis is damaging as it traumatizes and ostracizes the affected women. The treatment too is not favorable for most women in their reproductive age. The study further estimates the management of malaria and onchocerciasis through effective available anti-filaricide drug -Mactizen(Ivermactin) in these endemic areas to uplift the burden of Onchocerciasis in these women.

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SAFETY OF SURGICAL TREATMENT FOR CYSTIC ECHINOCOCCOSIS OF THE LIVER: PRELIMINARY EVALUATION OF THE LITERATURE (1980-2005)

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Surgery is still considered by many the main treatment for cystic echinococcosis (CE) of the liver. However, benzimidazole derivatives and percutaneous drainage are now available and the role of surgery should be redefined in the light of these alternative options. To our knowledge, no paper has addressed the comparison of therapeutic options in terms of safety. We screened the literature on open surgery for hepatic CE to assess its rate of complications and overall safety. We performed a Medline search of the literature in English using the key words "Echinococcal cysts", "Cysts" "Cystic Echinococcosis", "Liver Hydatidosis" and "Surgery" from 1980-2005. Papers on laparoscopic surgery were excluded. The authors' files were used as well. If the original article was not available, its abstract was used instead, if the number and location of treated cysts, together with major complications, was available. Data on 3465 surgical procedures were available from 13 papers and 33 abstracts. Demographics were available for 1026 male and 1348 female patients

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(mean age was 38.5). Of the 3465 procedures, only 485 (13.9 %) were radical (447 pericystectomies and 38 segmentectomies). Complications included 4 cases of lethal anaphylactic shock, and several cases of sepsis, biliary fistulas, cistobiliary rupture, bleeding, cholangitis, pancreatitis, and pulmonary embolism. Surgery for CE of the liver still carries a significant risk of complications. Radical surgery is considered the best option, but is performed only in a minority of cases. Serious complications are numerous, and no less than 4 episodes of lethal anaphylactic shock were recorded. In this review, surgery compares unfavourably with percutaneous treatments for hepatic CE in terms of safety, as reported previously. Further research is warranted in this area if a more rational approach to the choice of treatment for hepatic CE is to be taken.

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DOSE DEPENDENT EFFICACY OF SP FOR INTERMITTENT PRESUMPTIVE THERAPY OF MALARIA IN PREGNANCY AMONG HIV INFECTED ZAMBIAN WOMEN

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WHO recommends 2 courses of intermittent presumptive therapy (IPT) with sulfadoxine-pyrimethamine (SP) for reducing malaria in pregnancy during trimesters 2-3. Monthly SP is superior to standard IPT for HIV+ women; the optimal number of SP courses is uncertain. This is an "as treated" analysis of a recently completed placebo controlled, double-blinded study comparing standard 2-dose SP-IPT (S-IPT) vs. intensive monthly SP (I-IPT) among HIV infected women from Ndola, Zambia (mesoendemic[DHH1] for malaria). Although participants were randomized to either S-IPT or I-IPT, subjects actually received from 1-6 courses of SP (1500 mg sulfadoxine/75 mg pyrimethamine) by study end. We compared outcomes between 1 vs. ≥2 courses of SP. Outcomes were maternal hemoglobin (Hb), placental infection (% positive histology), birth weight (grams), low birth weight (LBW) (% <2500 g)[DHH2], infant cord blood parasitemia (% positive), and prematurity (% gest. age <37 weeks by Dubowitz). 391 women contributed data; placentas were obtained for 360 (91.4%). Treatments groups were similar in regards to age and gravidity. 34 women received 1 course of SP; 357 ≥ 2-courses; 178 ≥ 3; and 122 ≥ 4. Additional courses of SP showed graded benefits for all outcomes (Table); lower birth weight and infant prematurity were strongly associated with single course SP. 40.3% of babies born to mothers who had 1 course were LBW vs. 10.1-11.3% who had 2 or more courses (RR 3.6, 95% CI 2.2-6.0, P<0.01). 1 dose; 2 or more; 3 or more; 4 or more Maternal Hb -B - g/dL (n) 10.5 (30) 11.4 (321)* 11.4(154)* 11.5 (105)* Placental infection - % (prop.) 31.3 (10/32) 26.8 (88/329) 25.5 (41/161) 23.0 (26/113) Infant birth weight - grams (n) 2642 (32) 2962 (345)** 2986 (171)** 3015 (119)** Infant cord blood parasitemia - % (prop.) 6.9 (2/29) 1.8 (6/331) 0.6 (1/163)* 0.9 (1/115)* Gestational age < 37 weeks - % (prop.) 82.4 (28/34) 54.6 (195/357)** 53.9 (96/178)** 47.5 (58/122)** In conclusion, though our intent-to-treat analysis revealed no differences between S-IPT and I-IPT, this "as treated" analysis showed dose-dependent benefits of SP courses to mother and baby. Single course SP is inferior to multi-dose regimens and should not be used, even in areas with mild malaria transmission. Given the inferiority of single course SP-IPT, programs must ensure that HIV+ pregnant women at-risk of malaria receive at least 2 IPT courses of SP. (* P<0.05 for comparison vs. 1 dose of SP **P<0.01).

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USEFULNESS OF TELEDIAGNOSIS IN CONFIRMATORY LABORATORY DIAGNOSIS OF CASES OF PARASITIC INFECTIONS

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Laboratory diagnosis of parasitic diseases typically relies on identification of diagnostic morphologic features of parasites. The accuracy of morphology-based diagnosis depends on laboratorians' expertise in recognizing such features of the organism and the quality of the specimens being examined. Between 2002 and 2005, the parasitology reference diagnosis laboratory at the Centers for Disease Control and Prevention accepted 2498 specimens for diagnostic, or confirmatory, assistance. Approximately 21% of those requests were received at CDC as telediagnosis inquiries through the DPDx project; telediagnosis is the electronic transmission of digital images of suspected parasites in clinical specimens. To perform telediagnosis a laboratory must have a microscope, a digital camera, and a computer with Internet access. In this study we report data on DPDx telediagnosis activities from 2002-2005. During this period, DPDx processed 513 telediagnosis inquiries for diagnostic assistance, of which an average of 64% did not require additional examination or further testing for definitive identification of the parasite associated with the infection. An average of 22% required follow up by direct morphologic examination of the specimen through microscopy, and an average of 14% required PCR to achieve final identification of the parasitic agent at species level. The 160% increase in telediagnosis consultation during this period indicates a positive trend in the use of telediagnosis as a diagnostic tool in parasitology. The cost and time effectiveness of using telediagnosis for assistance will also be reported by comparison with the traditional ways of handing specimens. Telediagnosis is approximately 80% less expensive and faster, as diagnostic assistance can be provided within minute to hours through telediagnosis. We conclude that because parasitology remains a highly visual field, use of a remote technology approach such as telediagnosis can be extremely useful in confirmatory diagnosis.

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EFFECTIVENESS VERSUS COST OF FIVE NATIONAL SCALE ITN DISTRIBUTION SYSTEMS IN SUB-SAHARAN AFRICA

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Insecticide treated bed nets (ITNs) are an effective malaria control tool throughout sub-Saharan Africa. Policy makers and donor partners need better evidence regarding which ITN distribution system is the most efficient and sustainable. In order to generate comparable and policy-relevant evidence we aimed to review systematically five large national-scale ITN programmes: targeted subsidized distribution through ante-natal clinics (ANC) in Malawi; free distribution at the community level in Eritrea; EPI - linked free distribution in Togo; public-private partnership in Senegal; and finally an integrated national programme based on public-private partnerships and including discount vouchers delivered through Reproductive and Child Health clinics in Tanzania. Operational descriptions of the five national ITN programs in Sub-Saharan Africa were

compiled using a standardized framework. Costs were compiled for both the set-up and maintenance phases, using a standard costing framework developed for this purpose on the basis of existing guidelines. The emphasis was put on a financial analysis of the cost to the provider, but a full economic analysis was also performed. Further analysis will include a cost-effectiveness analysis using the effectiveness data from an existing systematic review. Results indicate that there are significant differences in cost per output (cost per ITN delivered, cost per person-year protection) for different strategies but that all distribution systems are highly attractive public health interventions by any standard. Startup costs are usually high due to the need for extensive training and intense promotion. Main sources of variation in cost were related to the type of targeting and to the cost of the nets. In conclusion, all different approaches lead to rapid increase in the level of protection afforded to the main groups at risk. Determinants of cost and implications for financing, health system capacity strengthening, equity and sustainability need to be further investigated.

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REVERSIBLE LYMPHATIC DYSFUNCTION CAUSED BY GNATHOSTOMA SPINIGERUM INFECTION

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Gnathostomiasis is an emerging infection that is increasingly seen in immigrants and in returning travelers. Historically, infection with *Gnathostoma* sp. had predominantly been reported from Asia, although there are significant numbers of reports of infection from South and Central America Classically, gnathostomiasis presents with non-specific, cutaneous and gastrointestinal symptoms, followed by eosinophilia and recurrent transient angioedema or creeping eruptions as the larva migrates. Here we report a case of gnathostomiasis that presented with stable bilateral lower extremity edema and lymphatic dysfunction. A 49 yo Cambodian-American woman developed diarrhea, vomiting, chills and fevers while visiting family in Cambodia. Hospitalized after her return to the U.S., she was found to have an absolute eosinophil count of 3900/mm^3 and an ESR of 70. A thorough infectious disease evaluation failed to identify an etiology. Her fevers and myalgias persisted despite treatment with trimethoprim-sulfa and mebendazole, although her GI symptoms gradually resolved. She then developed bilateral lower extremity edema, left greater than right, which persisted. Antifilarial IgG levels were strongly positive, but IgG4 levels and circulating filarial antigen testing for *Wuchereria bancrofti* were negative. Lymphoscintigraphy showed abnormal delay of lymphatic flow in the left leg. She was treated presumptively with diethylcarbamazine for a prepatent filarial infection. On follow-up, her symptoms had improved transiently, but then returned; her lymphedema and eosinophilia persisted. Gnathostomiasis immunoblot was found to be positive for the diagnostic 24kD protein. The patient was treated with a 3-week course of albendazole. On therapy, she developed several pruritic nodules. A small, motile worm morphologically consistent with a *Gnathostoma* emerged from one of the nodules. DNA was extracted from the worm, and a 283 bp PCR product was identified using consensus primers for the Gnathostoma 5S rRNA genes. Sequence analysis showed homology to *G. spinigerum* rRNA. On follow-up, her eosinophilia and lower extremity edema resolved. A repeat lymphoscintigraphy 4 months after treatment was completely normal. This patient demonstrates an unusual manifestation of gnathostomiasis with bilateral, lower extremity edema (nonmigratory) associated with reversible defects in lymphatic flow.

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THERAPEUTIC RESPONSE TO THIABENDAZOL, IVERMECTIN AND ALBENDAZOLE IN IMMUNOCOMPROMISE AND IMMUNECOMPETENT PATIENTS INFECTED WITH STRONGYLOIDES STERCORALIS

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The objectives of this study were: 1) Compare efficacy of thiabendazol (TBZ), ivermectin (IVM) and albendazole (ALB) in immunocompromise and immunecompetent hosts infected with S. stercoralis. 2) Identify risk factors associated to an inadequate therapeutic response. 292 patients with Strongiloidiasis were studied between 1989 y 2005, 193 immunocompetents y 99 immunocompromise hosts (38 HIV/AIDS and 61 with other immunsupresive disorder (mieloproliferative disorders, lymphoma, solid tumors and/or steroid therapy). 175 cases received TBZ (119 immunocompetents, 21 HIV/AIDS y 35 non-HIV immunocompromise hosts), 83 IVM (43, 15 y 25, respectively) y 34 ALB (31, 2 y 1, respectively). At enrollment, they underwent through clinical history and physical examination and provided stool specimens for identification of larvae through Baermann technique and agar plate culture. Parasitological cure: 88.4% (260/294); TBZ: 91%, IVM: 96.3% and ALB: 58.6%. There was no statistical difference between TBZ and IVM treatment for Strongiloidiasis (p=0.516). Patients who received IVM or TBZ were more likely to have parasitological cure than those who received ALB (OR_{IVM}: 50; IC 95% 2.4-1073; p=0.002). (OR_{TBZ}: 184; IC 95% 3,3-10.211; p=0.001). Patients with eosinophil counts <450/mm^3 at diagnosis associated to high parasitological burden (≥50 larvae pc) were less likely to have parasitological cure (OR: 0.02; IC95% 0.001-0.7; p=0.011). Similarly, among patients with high parasitological burden (≥50 larvae pc), those who have eosinophil counts ≥ 450/mm^3 at diagnosis were 13.5 times more likely to have parasitological cure than those that had < 450 eosinophils/mm^3 at diagnosis. In conclusion, TBZ and IVM are both good options for the treatment of Strongyloidiasis. HIV patients responded better to IVM than to TB, but there was not statistical difference. ALB is an inadequate treatment for Strongyloidiasis. Patients without eosinophilia, HIV/AIDS and other immunecompromised patients, have a higher chance of treatment failure when the parasitological burden is high.

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COMMUNITY USE AND IMPACT OF A SUPPLEMENTAL WEANING FOOD DURING A DIARRHEA AND MALNUTRITION OUTBREAK -- BOTSWANA, 2006

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Porridge is a primary weaning food for children in Botswana. Because fortified weaning foods can prevent malnutrition, a national program has provided free vitamin A-fortified sorghum-soy porridge to all children <5 years old since 1993. In response to a severe pediatric diarrhea and malnutrition outbreak in Botswana, we assessed the use of porridges in the community. From March 29 - April 12, 2006, we used two-stage random cluster sampling to survey households with children <5 years. Caregivers were interviewed about child health, diet history, and perceptions and use of fortified porridge; children were weighed and measured. We defined acute malnutrition as weight-for-height Z-score ≤ -2 or edema/kwashiorkor, and stunting as height-for-age Z-score ≤ -2. We examined porridge use for associations with diarrhea and malnutrition. We assessed 339 households with 537 children <5 years old. The median age was 28.3 (range 0-59) months; 265 (49.5%) were male. During the 24 hours before interview, 384 (74.1%) children had consumed any porridge and 58 (10.9%) had fortified porridge distributed by the

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feeding program. Since January 1, 2006, 170 (32.0%) children had at least one diarrhea episode, 35 (8.0%) were acutely malnourished, and 67 (15.8%) were stunted. Diarrhea, acute malnutrition, and stunting were not significantly associated with eating fortified porridge 24 hours or 7 days before interview. Among caregivers, 187 (58.8%) considered fortified porridge beneficial to children. Of those who did not, 102 (82.9%) believed it causes diarrhea and 11 (8.9%) that it makes children ill. Adult consumption of government-distributed fortified porridge intended for children <5 years old was reported in 247 (78.7%) households. In conclusion, despite free distribution, fortified porridge was regularly consumed by only one-tenth of children <5 years old and did not appear to protect against diarrhea or malnutrition. Many caregivers did not consider fortified porridge beneficial to children; instead, it is frequently consumed by adults. Further evaluation of fortified porridge distribution, preparation and practices may identify opportunities to improve the national feeding program.

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CLINICAL DIFFERENCES BETWEEN IMMUNECOMPETENT AND IMMUNECOMPROMISE PATIENTS INFECTED WITH STRONGYLOIDES STERCORALIS

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The objective of this study was to compare clinical symptoms and eosinophil count in immunecompetent and immunecompromise patients infected with *Strongyloides stercoralis*. 605 Strongyloidiasis cases were studied from 1989 to 2005, 398 were immunocompetent patients and 207 immunocompromise (65 HIV/AIDS and 142 with mieloproliferative disorders, lymphomas, solid tumors and/or steroid therapy.) At enrollment, they underwent through clinical history and physical examination and provided stool specimens for identification of larvae through Baermann technique and agar plate culture. Two retrospective studies were settled comparing: 1) Immunocompetent pacients vs. HIV/AIDS, and 2) Immunocompetent vs. Immunocompromise patients without HIV infection. HIV patients were less likely to present neither relative nor absolute eosinophilia ($OR_{relative}$ = 0,1; IC95%: 0,1-0,3; p<0,001; and, $OR_{absolute}$ = 0,1; IC95%: 0,1-0,2; p<0,001). Furthermore, they are more symptomatic than immunecompetent hosts (OR=6,4; IC95%: 1,9-21,0; p<0,001). Strongyloidiasis in HIV/AIDS patients is strongly associated to diarrhea (OR=4,8; IC95%: 2,2-10,6; p<0.001), chronic diarrhea (OR=4,5; IC95%: 2,0-10,5; p<0.001), loose stools (OR= 15,4; IC95%: 1,9-125,0; p=0,001), more than 5 stools per day (OR=3,5; IC95%: 1,1-10,8; p=0,001) and weight loss (OR=6,4; IC 95%: 3,2-12,6; p<0,001). Non-HIV/AIDS immunecompromised patients complain less of abdominal pain and are less likely to develop eosinophlilia ($OR_{absolute}$ = 0,4; IC95%: 0,2-0,8; p=0,005) como ($OR_{relative}$ =0,4; IC95%: 0,2-0,8; p=0,011). In conclusion, *S. stercoralis* is an important cause of diarrhea and wasting syndrome in HIV/AIDS patients in developing countries. Almost 40% of HIV/AIDS patients do not develop eosinophilia ≥ 5% and 64.6% have < 450 eosinophils/mm^3. This parasite should be suspected in immunecompromise hosts coming from endemic areas even in absence of eosinophilia. IC_{95}

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THE SPECIFIC GENOTYPE OF INDONESIA CHIKUNGUNYA VIRUSES ISOLATED FROM 1983-2001

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Chikungunya (CHIK) virus transmission has been confirmed in Indonesia on the islands of Kalimantan, Sumatra and Java since1983 with the most recent outbreak occurring in April of 2006. Here, we report the partial sequencing of the E1 envelope glycoprotein gene from CHIK viruses isolated from human and animals sampled during periodic outbreaks between 1983 - 2001. CHIK viruses were passaged in C6/36 cell lines, Aedes sp. or Toxor sp. Mosquitoes, purified and then sequenced. Phylogenetic trees generated using both parsimony (1000 bootstrap replicate) and likelihood methods (PAUP) had identical topologies. Indonesia CHIK isolates clustered together in a specific branch disparate from other strains of Asian genotype CHIK viruses with a high genetic similarity (97-100 %). The genetic distance of Indonesian isolates compared to Asian genotype, West African, Central/East African, and O'nyong-nyong (ONN) virus was 0.784-1.07, 0.996-1.133, 0.951-1.172, and 0.941-1.196, respectively using Jukes-Cantor statistics. Interestingly, our results demonstrate that viruses isolated from humans and animals were identical. This sequence characterization suggest that Indonesian CHIK viruses differ from Asian genotype viruses. To our knowledge, this data provides the first account of CHIK virus sequences from Indonesia strains and has implications for vaccine development.

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EPIDEMICS OF AN "ENDEMIC" MYCOSIS: A SUMMARY OF FOCAL OUTBREAKS OF COCCIDIOIDOMYCOSIS 1940-2004

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Much of what is known about the epidemiology of coccidioidomycosis has been accrued in skin test and sero-surveys. Although single-source outbreaks of this infection exist, this body of work has not been previously studied in aggregate. New insight into the epidemiology of coccidioidomycosis may be gained from structured analysis of these case clusters. A literature search using keywords "coccidioidomycosis" or "Coccidioides" or "valley fever" NOT "Rift," AND "epidemiology" or "epidemic" or "outbreak" was conducted. The search utilized MEDLINE, Google, BIOSIS, SciElo, and LatinDex, without restriction to humans or English language. Reference lists of pertinent works were also examined for those describing point source or focal case clusters. Healthcare-associated and multi-year outbreaks were excluded. From 3140 titles examined, 39 citations describing 32 focal outbreaks were located, with publication dates from 1942-2004. Frequency of published outbreaks increased after 1995 (p=0.05), when coccidioidomycosis was added as a nationally notifiable disease at the southwest regional level. From reports with evaluable data, median outbreak size was 15 cases, with mean attack rate 66%, and incubation period 11.2 days. Anthropogenic soil manipulation was associated with 23 (72%) of the outbreaks (e.g. scientific excavations, construction, children playing). The largest outbreaks were associated with natural events (dust storm, earthquake/landslides). Twenty- six (81.2%) outbreaks originated in California. Groups traveling into endemic areas accounted for 18/32 (56.2%) outbreaks. Of the 32 outbreaks, 9 (28.1%) occurred in areas hitherto unknown to be endemic for Coccidioides. In conclusion, although classically labeled an "endemic" mycosis, coccidioidomycosis prominently causes focal, point-

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source outbreaks. These case clusters illustrate important features of this emerging fungal infection.

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RECURRENT ASEPTIC MENINGITIS DUE TO CYSTICERCOSIS

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Cysticercosis is a larval infection acquired by ingesting Taenia solium eggs. The presentation of neurocysticercosis (NC) depends on the number, activity and location of cysts and on the host's immune response. Cysts frequently locate in the subarachnoid space, causing arachnoiditis and obstructive hydrocephalus, and their disintegration causes an inflammatory reaction and abnormal cerebrospinal fluid (CSF) findings. Serologic tests are important for diagnosis, especially for meningeal disease. We present a case of recurrent meningitis where CSF serological tests helped define recurrence of NC. A 45-yr.-old Colombian woman presented with recurrent meningitis over 3 months. She was diagnosed with NC 3 yrs. previously when she presented with headaches, seizures and corroborating computed tomography (CT) findings. She improved with albendazole and steroids, and magnetic resonance imaging (MRI) 2 yrs. later was normal. She presented again in Dec. 2005 with headaches for 1 wk. and neck stiffness but no other symptoms. Physical examination (PE) was normal. Lumbar puncture (LP) revealed high opening pressure, low-grade pleocytosis with lymphocytic predominance and hypoglycorrhachia. Broad-spectrum antibiotics and acyclovir were given. No other infectious agent was found, and the diagnosis of NC was considered because of positive CSF serologic testing. She returned 2 months later with similar symptoms, a normal PE, and LP nearly identical to the prior one. MRI at that time revealed abnormal meningeal and multifocal cortical enhancement thought to be secondary to inflammatory cysticercosis and ruptured cysts; another MRI 2 months later showed larger cysts in the subarachnoid space and suspicion of increased leptomeningeal involvement. Quantitative serologic tests for cysticercosis in CSF were markedly elevated. In conclusion, cysticercal meningitis can present with elevated intracranial pressure and other neurologic signs. The CSF analysis may appear as aseptic meningitis, tuberculous meningitis or malignancy. Serologic tests are important tools in cases of aseptic meningitis, especially when symptoms recur and no definite etiology can be established. CT and MRI can be adjuncts in establishing the diagnosis of NC. Cysticercosis should be considered as a cause of aseptic meningitis in patients from endemic areas, and serologic studies should be performed when symptoms continue or recur.

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LEISHMANIASIS AND HIV CO-INFECTION IN NORTHCENTRAL VENEZUELA

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Leishmania species can cause a wide spectrum of cutaneous disease in HIV-positive patients: from cutaneous (CL), mucocutaneous (ML), diffuse cutaneous or post-kala-azar, and also visceral leishmaniasis (VL). We evaluated the epidemiologic features of leishmaniasis in a series of HIV-positive patients from Northcentral Venezuela from 2000 to 2006. A total of 6 patients with the clinical diagnosis of leishmaniasis and HIV-infection were evaluated at our referral center. Different diagnostic methods were used to confirm the diagnosis (Montenegro Skin Test, MST; Indirect Immunofluorescence Test, IIF; and smear). Clinical and epidemiological features of leishmaniasis among these patients were evaluated. Mean age was 28 years of age, (range 3 to 41 years); 83% were males and 17% were females; 33% were from Sucre State, with a mean clinical evolution of 8 months. From these patients, 66% (4/6) were CL, 17% (1/6) were ML and 17% (1/6) were VL. From those patients with CL, most of them presented just one lesion (75%, 3/4), all of them were anergic to MST. Co-infection of HIV and leishmania species in Northcentral Venezuela is rare. However, it tends to be associated with either disseminated cutaneous or visceral leishmaniasis. The use of skin testing for leishmaniasis is not clinically helpful in patients with HIV-infection.

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THE WIDAL TUBE DILUTION TEST EVALUATION AMONG TYPHOID FEVER PATIENTS IN JAKARTA, INDONESIA.

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Microorganism isolation through culture is the gold standard in diagnosis of typhoid fever. However this procedure takes two to seven days. In the interim the attending physician requires some laboratory evidence in the management of the patient, sometimes with high fever and altered mental status. To evaluate the value of single Widal test, we conducted a serological evaluation of 129 febrile patients admitted into a community-based prospective study of enteric fever in North Jakarta, Indonesia. The culture testing of these patients showed that they included 67 typhoid fever patients, 3 paratyphoid A patients and 1 patient with a positive blood culture for Salmonella group C; the rest had negative blood culture. Using a cut-off of ≧1:40 for O antigen, the Widal test had 72% sensitivity, 82% specificity, positive predictive value of 82% and negative predictive value of 71%; the corresponding values for the H antigen were 35% sensitivity, 100% specificity, 85% positive predictive value and 55% negative predictive value. At a cut-off of ≥1:80, for O antigen, this test had 51% sensitivity, 96% specificity, positive predictive value of 94% and negative predictive value of 62%; the corresponding values for the H antigen were 35% sensitivity, 100% specificity, 100% positive predictive value and 57% negative predictive value. There was no cross reactivity of Widal test results, except that 2 patients with S. paratyphi A positive culture results had positive values of 1:80 and 1:160 for O antigen. Early management of typhoid fever in specific populations may benefit from a single Widal test in the acute phase, if an appropriate cut-off titer for that population has been determined.

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CUTANEOUS LEISHMANIASIS IMPORTED FROM COLOMBIA INTO NORTHCENTRAL VENEZUELA: A REVIEW OF 29 CASES

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Imported leishmaniasis could be defined as any case acquired outside of a defined area in which the diagnosis of leishmaniasis is made. This definition has been used to refer to the diagnosis of a disease in a patient who comes from an endemic area and displays symptoms or seeks medical attention in a nonendemic zone. However, this phenomenon can also occur between two endemic zones. We evaluated the epidemiologic features of imported cases of cutaneous leishmaniasis (CL) that come from Colombia into Northcentral Venezuela from 2000 to 2006. A total of 29 patients with the clinical diagnosis of CL proceeding from Colombia were evaluated at our referral center (IMT). Different diagnostic methods

were used to confirm the diagnosis (Montenegro Skin Test, MST; Indirect Immunofluorescence Test, IIF; and smear). Clinico-epidemiological features of CL among these patients were evaluated. Mean age was 35 years old (age range was 7-64); 55% were males and 45% were females; there was a considerable dispersion in the places of origin (all from north departments of Colombia), 7% were from Sucre Department (in northwest), 3% from Antioquia Department (in west), 3% Magdalena Department (in north), 3% from Santander Department (in northeast), 3% from Bolivar Department (in the west), among other. These patients presented a mean clinical evolution of 3 months. Most patients presented just one lesion (17%), which were located mostly in extremities (20%). Of the 29, 11 (38%) patients were positive by MST, 41% were positive by IIF and 7% were positive by smear. MST mean was 13 mm. The median titer for IIF was 1/128. The identification of imported CL in our setting becomes important, given the differences in the epidemiology of the disease and the clinical severity of leishmaniasis between both zones (ecological characteristics, circulating Leishmania spp., and population characteristics) and the risk of mucocutaneous forms of the disease.

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HEPATITIS E INFECTION IN THAI TROOPS DEPLOYED WITH UNITED NATIONS PEACEKEEPING FORCES

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Hepatitis E Virus (HEV) causes large epidemics of acute and sporadic hepatitis in Asia, the Middle East, and parts of Africa and Mexico. Epidemics are frequently associated with fecal contamination of drinking water. Death due to fulminant hepatitis may result from HEV infection and the mortality rate ranges from 1-2% among the general population to 20-30% in pregnant women. Currently there is no vaccine commercially available for hepatitis E. Several outbreaks of hepatitis E have been reported in military environments which led to significant loss of soldier duty days. Between October 99 and December 2005, Thai soldiers were deployed to Timor-Leste (n = 5140), Afghanistan (n = 109), Iraq (n = 879) and Burundi (n = 174) as part of multinational forces for the United Nations in its peace keeping efforts. Effective disease surveillance does not exist in any of these countries. The objective of this study was to determine the antibody pattern to HEV in serum over time using a sensitive ELISA technique. Cases of hepatitis E were not reported during the deployment. Background prevalence of anti-HEV in this military population was 19.6, 20.2, 21.3 and 28.3% for troops deployed to Timor-Leste, Afghanistan, Iraq, and Burundi, respectively. The seroconversion (as defined by a 4-fold rise in antibody titer) rate per year was 1.9, 5.6, 5.6 and 4.8 % respectively. These were not significantly different. A vaccine to protect against hepatitis E would be an important adjunct to prevent this important disease which can cause high morbidity among military personnel.

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STUDIES ON SOME NUTRITIONAL FACTORS IN THE SEVERITY OF VISCERAL LEISHMANIASIS

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Visceral leishmaniasis(VL) or Indian kala-azar is a protozoan disease caused by the parasite Leishmania donovani.The disease is pandemic in eastern part of India and is a major public health proble.Since the disease predominantly affects the people of low income group in whom the nutritional status is very poor and malnutrition has been considered as a major risk factor for the development of VL. This individual risk factor has been considered as one of the important factor in the incidence of the disease also. Children are at great risk of developing VL when they are malnurished. The relationship between malnutrition and VL is poorly understood. The various nutritional laboratory related tests have been carried out in different categories of malnurished VL patients. The preliminary study reflects that as malnurished index increases, there is upregulation of triglyceride. Hypo-cholesterolemia have been observed in VL infection. Apolipoprotein A1 downregulation has also been observed. The preliminary study advocates that during leishmania infection there may be replishment of cell membrane cholesterol and it may help the parasite in the establishment of the infection within the macrophage.

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CLINICOPATHOLOGICAL CHANGES IN DERMAL LESIONS OF POST KALA AZAR DERMAL LEISHMANIASIS (PKDL) CASES IN BIHAR, INDIA

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Post kala azar dermal leishmaniasis (PKDL) is increasingily being recognized in Bihar, India as a cutaneous complication of visceral leishmaniasis. Since it is considered an important factor in kala azar transmission, its early detection, diagnosis and assessment of effective treatment is very important for disease control. In our present study on 30 PKDL cases, 82 % had past history of kala azar treatment. The disease presented with hypopigmented macular lesions on the body with or without papular, nodular and erythematous lesions predominantly over face. Skin biopsies to prepare imprint smears were collected superficially from dermal lesions of these PKDL cases. Leishmania parasites were demonstrated microscopically in imprint smears from 93% of papulonodular and erythematous and 40 % of macular lesions. Negative cases were diagnosed on the basis of past history of VL, distribution of skin lesions, their histopathological changes and DAT positivity. Microscopical examination of cellular infiltrates of biopsy imprint smears from PKDL lesions demonstrated 25-300/OIF mononuclear cells consisting of predominant histiocytes with vacuolation, many lymphocytes, some plasma cells and Leishmania amastigotes of varying density. After schedule treatment with Sodium Antimony Gluconate (SAG), the papulonodular lesions cleared clinically but the pathological changes persisted in the imprint smear of many cases with presence of mononuclear cells 20-200/ OIF. Therapeutic response of the macular cases was poor and persisted both clinically as well as pathologically. They required prolonged treatment with SAG. Further study on PKDL cases can assess the effectivity of the treatment either as a complete disappearence of lesions or any relapse.

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THERAPEUTIC EFFICACY OF AMODIAQUINE, SULFADOXINE/ PYRIMETHAMINE, AND COARTEM® IN CHILDREN WITH UNCOMPLICATED FALCIPARUM MALARIA AT BUTIMBA SENTINEL SITE IN MWANZA, TANZANIA

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In 1999, baseline data from children aged 6-59 months treated with sulfadoxine/pyrimethamine (SP), in a 14-day *in vivo* test, revealed only 7% treatment failure at Butimba sentinel site in Mwanza, Tanzania. The

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data together with other from 7 more sites were utilized in changing first line antimalarial drug from chloroquine to SP in 2001. The current study to assess the efficacy of SP, amodiaquine (AQ), and Artemether/Lumefantrine (Coartem®) was conducted at Butimba in 2005. The main aim was to obtain more data on *Plasmodium falciparum* response to the 3 drugs. Coartem® is earmarked to become first line antimalarial drug in Tanzania in 2006. Children who met the inclusion criteria were randomized to receive one of the 3 drugs (SP=64, AQ=64, Coartem® =83). Filter paper bloodspots were collected for PCR parasite genotyping. SP was given as a single dose equivalent to 1.25mg/kg body weight with respect to pyrimethamine whilst AQ was given in 3 divided doses totalling 25mg/kg body weight (over three days). All daily doses of SP and AQ were supervised. The first dose of Coartem® and the daily doses for the next 2 days were administered under supervision. Successful 28 day follow-up was made in 61, 62, and 74 cases on SP, AQ, and Coartem® respectively. Evaluation of data based on 28 day follow-up showed adequate clinical and parasitological response (ACPR) of 54.1%, 80.6% and 83.8% in the SP, AQ, and Coartem® treatment arms respectively. There were no cases of early treatment failure (ETF) with Coartem® whilst highest ETF rate was in the SP arm at 18.0%. Overall late treatment failures (LTF) were: SP= 27.9%, AQ=17.7%, Coartem® =16.2%. By day 14 of follow-up, ACPR was 68.9%, 91.9% and 97.3% in the above treatment arms; indicating that most failures occurred later on. These results with other data have been used in reviewing anti-malarial drug policy in Tanzania. We conclude that SP is no longer effective and Coartem® is a promising alternative in the area. AQ should be considered in fixed dose combination. PCR corrected data to confirm true LTF cases will be presented.

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THE EFFECTS OF STUDY ENROLLMENT, BEDNET USE, AND CURATIVE THERAPY ON MALARIA INFECTION, ANEMIA, AND GROWTH IN YOUNG GHANAIAN CHILDREN

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Bednets and intermittent S/P therapy protect young African children from malaria infection, illness and death. Quantitative evidence of these benefits is not easily acquired. We hypothesized that bednet use would also be associated with better/faster growth in this age group, and that curative treatment during the high malaria transmission period would further enhance health and development. Random sampling of 646 children 6-24 mos. old in a non-irrigated sector of northern Ghana during August, 2001, found no evidence that bednet use was associated with fewer/lower parasitemias, higher Hb, or better Z scores for growth. Non-febrile breast-feeding children of informed, consenting parents were enrolled and randomized to receive either supervised S/P + quinine curative treatment (n=251) or a vitamin D placebo (n=247). Malaria infections, Hb, and anthropometric measures were recorded at baseline and at regular intervals. During 16 weeks of follow-up (Aug.-Dec., 2001) slide-confirmed malaria accounted for 68% and 70% of illnesses, respectively, in the treated and placebo groups. Between group comparison at endpoint showed an expected delay to first symptomatic parasitemia in the "cured" group but an unexpectedly better profile of S/P effect for treatment of uncomplicated malaria cases in the placebo group. Within group comparison between baseline and endpoint identified significant improvements in Weight-for-Age (WAZ) and Height-for-Age Z (HAZ) scores in both groups that were independent of bednet use or initial cure. Notably, WAZ and HAZ change in girls improved significantly over that of boys. Mean Weight-for-Height Z scores, WAZ, and HAZ of enrolled children were significantly improved over those of a closely matched cohort of non-enrolled children. Children in both study arms achieved improved Hb and growth independent of bednet use or malaria treatment. Concurrently, despite rapidly falling Anopheles biting rates, non-enrolled children experienced lowered Hb levels, heightened parasitemia, worsened Z scores, and greater mortality. Study participation was protective and beneficial in both treatment and placebo groups.

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MICROSPHERE ASSAY FOR RELIABLE IDENTIFICATION OF CRYPTOSPORIDIUM HOMINIS AND CRYPTOSPORIDIUM PARVUM IN STOOLS

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Cryptosporidium hominis and *C. parvum* are associated with massive diarrhea outbreaks worldwide. Because these two species have different transmission cycles, species-specific identification in clinical samples may provide laboratory data of crucial importance in certain epidemiologic investigations. To date, the most reliable way to differentiate *C. hominis* and *C. parvum* is based solely on PCR amplification of specific genes followed by DNA sequencing analysis of amplicons produced. Despite its usefulness, this approach is labor intensive and time consuming when compared with DNA-based molecular methods that do not require sequencing analysis for species-specific identification and which have been used recently for identification of a number of different pathogens. In this study we describe a novel Luminex assay that can differentiate *C. hominis* from *C. parvum* in a reliable manner. This assay relies on DNA hybridization probes linked to carboxylated Luminex microspheres that hybridize to a specific complementary region of biotinylated PCR-amplified *Cryptosporidium* sp. microsatellite-2 region (ML-2) fragments where *C. hominis* and *C. parvum* differ by a single nucleotide substitution. The test was 100% specific when tested on a total of 40 DNA samples extracted from stools that were evaluated by microscopy-based direct fluorescent antibody test (DFA) and characterized at species level (i.e., *C. parvum* or *C. hominis*) by DNA sequencing analysis after PCR amplification of at least one of the genetic markers known to discriminate these two species. Mixed infections were also detected in 2 of the samples analyzed in this study. As few as 10 oocysts per 300 microliters of sample processed can be detected using this assay as determined by amplification of DNA extracted from stool samples spiked with different concentrations of *C. parvum* oocysts.

(ACMCIP Abstract)

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IMPAIRED ABILITY TO DOWN MODULATE THE IMMUNE RESPONSE

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Leishmania braziliensis is the causal agent of cutaneous leishmaniasis (CL) and mucosal leishmaniasis (ML). CL is characterized by one or multiple ulcers and ML is a severe complication of CL involving the nasal mucosa, palate and pharynx. The protective immune response to leishmania spp is mediated by IFN-γ and TNF-α production and induction of microbicidal products from macrophages. However, the immune response may also play an important role in the pathology of the disease. In the present study we correlated the immune response, as determined by measurement of cytokines in supernatants of mononuclear cells, as well as cellular activation markers of tissue damage. Moreover, the mechanisms involved

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in the exaggerated type 1 immune response observed in CL and in ML were evaluated. A correlation was found between production of pro-inflammatory cytokines and severity of clinical infection. There was a direct correlation between IFN-γ as well as cell markers of activation and increase in lesion size. The exaggerated T cell response in CL and ML was associated with a strong type 1 immune response as well as IL-17 production. This biased immune response was not appropriately modulated by IL-10 and CTLA-4. IL-10 fail to inhibit TNF-α and IFN-γ production in CL and ML patients and expression of IL-10 receptor was decreased in ML. This study extends our previous observation on the participation of pro-inflammatory cytokines and T cells in the pathogenesis of CL and ML. Furthermore, it supports the use of antimony combined with molecules that down modulate the immune response in the treatment of CL and ML.

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CHARACTERIZATION OF ONE OUTBREAK OF TYPHOID FEVER IN APARTADÓ- ANTIOQUIA, COLOMBIA

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On May 2005, an outbreak of typhoid fever was detected in Apartadó, a city of Antioquia state from Colombia. Thirty four patients showed typhoid fever clinical symptoms. Blood, and stool samples from 15 patients were taken for microbiological studies; also Salmonella was search in water samples from the area. The aim of this study was characterize phenotypic and genotypically, isolates from an outbreak of typhoid fever. To diagnose typhoid fever in patients, clinical examination, bacteriological cultures, and Polymerase Chain Reaction (PCR) to detect the hilA gene specific of Salmonella were performed in blood and stool samples. Additionally phenotypic and genotypic tests were carried out to bacteriological isolates, biochemical identification, susceptibility and resistance to antibiotics and detection of the presence of the hilA and invA genes from Salmonella pathogenicity island -1 (SPI-1). IS-200 was performed, with the aim to characterize the isolates and to confirm the clonal line. Samples from polluted water from a distribution channel were taken for culture using Readycult® Coliforms 100 (Merck Darmstadt, Germany). Also PCR for hilA gene detection was performed in water samples. The clinical diagnosis was confirmed in 15 patients, where Salmonella Typhi was isolated from blood and stool samples. All isolates were sensitive to 10 antibiotics tested; also they were positive to hilA and invA genes. The insertion sequence IS-200, showed a 700 bp in the 15 isolates studied. The phenotypic and genotypic tests confirmed common clonal origin of the Salmonella Typhi isolates in this outbreak, however clonal relation by genotyping requires confirmation. Water samples were positive for coliforms, but Salmonella was not isolated. PCR for hilA was negative in water samples. The molecular techniques helped to clarified that several strains of S. Typhi were circulating in environment and were responsible of this outbreak. Antibiotic resistance is not present in S. Typhi isolates from Colombia. The source of the infection could not be determined.

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COMPARISON OF REAL-TIME PCR PROTOCOLS FOR DETECTION OF CYCLOSPORA CAYETANENSIS IN STOOL SAMPLES

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Cyclospora cayetanensis is a coccidian parasite associated with diarrhea disease. Humans get infected by ingesting oocysts from contaminated food or water. In USA, foodborne outbreaks of cyclosporiasis have occurred almost every year since 1996, associated with consumption of fresh produce. Detection of the oocysts in stained stool smears or in wet mounts by UV fluorescence microscopy are the gold standards for laboratory diagnosis of cyclosporiasis. However, these methods cannot identify *C. cayetanensis* on species level, and molecular analysis is needed for this purpose. Some PCR tools have already been described, but no comparative data of these assays is available yet. In this study, we compared three real-time PCR tests for the specific detection of *C. cayetanensis* in stool: one nested multiplex SYBR Green assay and two TaqMan assays previously published (TaqMan1 and TaqMan2), which all target the 18S rRNA gene. The SYBR Green assay used species-specific primers in a multiplex format to simultaneously amplify and distinguish *C. cayetanensis* from the simian *Cyclospora* species, i.e., *C. cercopitheci, C. colobi,* and *C. papionis*, as well as *Eimeria* species. The TaqMan assays were designed for the detection of *C. cayetanensis* only. A total of 116 stool samples were used to compare the assays. As determined by microscopy and a nested conventional PCR assay, 48 were positive for *C. cayetanensis* and 39 contained other intestinal parasites, including the simian *Cyclospora* species listed above and *Eimeria tenella*. In the remaining 29 samples, no parasites were detected. In our hands, the SYBR Green assay performed the best, with specificity and sensitivity of 100% and 90%, respectively. However, because it was a nested PCR assay it was time-consuming and prone to amplicon contamination. The TaqMan2 assay had the same sensitivity as the SYBR Green assay but it occasionally produced false positive results and could not accurately differentiate between simian and human *Cyclospora* species (88% specificity). The TaqMan1 assay displayed low amplification efficiency, poor sensitivity (73%) and occasionally produced false positive results (90% specificity). The results of this comparative study should be of value for laboratories that plan to implement molecular tools for laboratory diagnosis of cyclosporiasis.

(ACMCIP Abstract)

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TICKS INFECTED WITH RICKETTSIA IN VILLETA, CUNDINAMARCA, COLOMBIA

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In 1936, an outbreak of Rocky Mountain spotted fever occurred in Tobia, Cundinamarca, Colombia. At that time, three species of hard ticks (Ixodidae) were known as vectors of the disease, namely ***Rhipicephalus sanguineus, Dermacentor nitens***, and ***Amblyomma cajennense***; however, one genus of Argasidae (***Ornithodorus***) was considered the most likely vector of Tobia fever despite the fact that rickettsiae were not identified in any of the specimens collected. Since then, no tick-directed studies have been conducted in the area. We recently reported three

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fatal cases of Rocky Mountain spotted fever in Villeta, 5 miles away from Tobia. Given that no current information is available about the tick species present in this area and whether they are infected with rickettsiae or not, we performed the study reported herein in which we identified the species of ticks present in this region and screened them for the presence of rickettsiae.

We collected a total of 679 adult ticks in the vicinity of the location where fatal cases were reported using dragging-flagging and direct collection from domestic animals (particularly dogs and horses). We identified the ticks at the species level and performed hemolymph test to select speciments for DNA extraction. We amplified two rickettsial genes, gltA and rOmpA, by PCR using primers pairs CS78-CS323 and RR190.547F-RR190.701R. Four species of ticks were identified: *Rhipicephalus sanguineus* (39.46%), *Anocentor nitens* (formerly *Dermacentor nitens*, 24.88%), *Amblyomma cajennense* (22.68%) and *Boophilus microplus* (12.96%). Of these, one R. sanguineus (0.37%), seven *A. cajennense* (4.54%) and one *B. microplus* (1.13%) were positive for rickettsial DNA. We conclude that the tick species previously reported are still present in the area, and that at least three of them are likely to be infected with Rickettsia. Moreover, they have been reported as possible vectors of spotted fever group rickettsioses in other endemic areas of South America.

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VECTOR-PATHOGEN SPECIFICITY OF BACTERIAL GUILDS MAINTAINED BY DOG TICKS AND LONE STAR TICKS

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Rocky Mountain Spotted Fever (RMSF), tularemia, and ehrlichiosis are frequently reported in the central and southern U.S. The main tick vectors for these agents are dog ticks and Lone Star ticks (LST). These ticks often co-occur and may feed on the same vertebrate hosts as adults, but usually do not share hosts in the larval and nymphal stages of development. Host-switching is thought to be an important mechanism in the emergence of zoonoses. The focal co-occurrence of LST and dog ticks and the availability of vertebrate hosts may increase the frequency of host-sharing among these two tick species thereby providing the opportunity for host-switching events. The agent of tularemia, for example, is said to be transmitted by either tick, suggesting some degree of host-sharing. To determine the frequency with which host-switching occurs with tickborne pathogens, we compared the prevalence of co-infection of various bacteria endemic within the southcentral U.S. which might be acquired during bloodmeals by dog ticks or LST such as *Francisella tularensis* and *Francisella* spp.; spotted-fever group (SFG) rickettsia; *Anaplasma phagocytophilum*; and *Ehrlichia chaffeensis*. Ticks were collected from twelve locations in four counties in Missouri and one county in Kansas. We analysed a total of 675 LST, and 95 dog ticks. DNA was extracted from tick homogenates and screened by PCR using primers targeting the SFG rickettsia ompA gene, A. phagocytophilum-specific 16s rDNA and *F. tularensis* fopA gene. Other agents will be detected using species-specific 16s rDNA primers. All amplicons will be sequenced. *F. tularensis* was detected in only one LST pool (0.9%), which was not coinfected by SFG rickettsia or *A. phagocytophilum*. SFG was detected in 67% of dog tick pools and 98% of LST pools. No evidence of a significant frequency of host-switching by dog tick and LST-maintained bacterial pathogens was detected, although further analyses are underway. Host-switches are likely to be rare events, but the great diversity of bacteria associated with dog ticks and LST may allow, by analysis of co-infection rates, a better estimate of the frequency of this phenomenon.

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OPTIMIZATION OF DENGUE SERONEUTRALIZATION ASSAY: NEW FORMAT ASSAYS AND CRITICAL PARAMETERS

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In vitro evaluation of functional Dengue antibodies is routinely assessed through quantification of serum antibodies that are able to inhibit viral growth on mammalian or insect cells. As more vaccine candidates are shifting progressively from pre-clinical to clinical proof-of-concept, this assay is becoming the major tool to establish correlates of protection in humans. Neutralization assay is usually performed as plaque reduction neutralization 50 (PRNT50) assay, in 6-, 12-, or 24- wells plaques, and is time and labor consuming. In this work we report the development of 2 alternate micro-neutralization assays in 96-well format plates, one based on miniaturization of the current PRNT50 assay (µPRNT50), and the other based on calculation of end-point limit dilution (SN50). We have also addressed here the question of the impact of the quality of the challenge virus preparation on the seroneutralization titer of a test serum sample. Viral suspensions containing the same quantity of infectious virus, but different amounts of particles were prepared, and the neutralization titer of several sera from monkey or mouse origin was established in a standard PRNT50 assay, againt these different preparations. Up to 3-log difference in the titer of the same serum was observed, according the virus preparation used. These data emphazise the importance of characterizating challenge virus preparations in order to get reproducible data.

(ACMCIP Abstract)

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EVALUATION OF A DRY-FORMAT GROUP-SPECIFIC REAL TIME REVERSE TRANSCRIPTASE-PCR ASSAY FOR DENGUE VIRUS

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Dengue (DEN) virus has reemerged at an alarming rate during the past two decades throughout tropical regions of the world to become the most important arboviral disease in terms of morbidity, mortality, and economic cost. Rapid identification of new infections is critical for effective disease control measures. However, current diagnostic methods based on antibody detection and/or the isolation of virus from serum samples are labor intensive, time consuming, and usually results become available after the patient has recovered from disease. We have developed a rapid, quantitative real-time RT-PCR (rRT-PCR) assay in dry format for the group-specific detection of DEN virus in human clinical samples. The assays were manufactured as dry reagents and all assay components including primers, probes and enzymes were dried directly into the reaction tubes. Dry assays were rehydrated immediately before use with a buffer supplied with the assays. RNA was extracted from test samples using the QIAGEN QIAamp viral RNA mini kit. The rRT-PCR assay was performed on the Cepheid, Smart Cycler® and consisted of a 20 minute RT step, linked to a 45 cycle PCR at 95°C and 60°C. The group assay detected known titers of DEN-1 and DEN-2 to dilutions of 10^{-1} plaque forming units (PFU)/ml, and detected DEN-3 and DEN-4 to 10^{1} PFU/ml. No inhibition was observed when DEN virus was spiked into normal human serum, and as little as 20µl of spiked human serum at 10^{2} PFU was detected. The assay specificity was determined to be 100% using an extensive cross-reactivity panel including related flaviviruses and normal human serum samples. Human clinical samples from Indonesia and Peru are being tested in comparison

with the standard viral isolation method. Preliminary results are promising and highlight the potential for the DEN RT-PCR assays as a tool for the epidemiological and diagnostic investigation of DEN fever. The dry assay format is stable at ambient temperatures and can be run on a portable instrument, potentially supporting remote laboratory testing in tropical regions.

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REPLICATION AND TEMPERATURE-SENSITIVITY PROFILES ON VERO, HUH-7 AND HEPG2 CELLS, OF, EITHER DENGUE VIRUS ATTENUATED STRAINS, OR CHIMERIC YF VIRUSES PRESENTING DENGUE ENVELOPE, COMPARED TO WILD-TYPE DENGUE VIRUS STRAINS

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The liver is clearly involved in Dengue (DEN) virus infections of humans. Transient elevation in alanine and aspartate aminotransferases levels is observed in the majority of DEN virus-infected patients, with a significant increase in the sera of patients with the more severe forms (DEN Hemorrhagic Fever/DEN Shock Syndrom, DHF/DSS). In addition, disease severity has been shown to be positively correlated with the magnitude of virus replication in hepatocytes. In an attempt to develop an *in vitro* model for evaluation of DEN virus hepatotropism, we have established the growth curves, on 2 different human hepatic cell lines, Huh-7 and HepG2 cell lines, and at 2 different temperatures, 37°C and 39°C, of several attenuated or chimeric DEN viruses having demonstrated inocuity in clinical and/or pre-clinical studies. The wild-type DEN virus strains from which these viruses were initially derived were used as references. Cells were infected à M.O.I. 0.01, and virus replication was quantified by qRT-PCR at regular intervals in the supernatant of infected cells. Different kinetics of replication were observed with the same virus on the 2 hepatic cell lines, the HepG2 cells being the less permissive ones to infection. Growth curves in Huh-7 cells, in most cases, were parallel to growth curves in Vero cells. A clear restriction of growth at 39°C was non surprinsingly observed with low-temperature adapted strains, both in Vero and Huh-7 cells, but was not a characteristic of chimeric viruses. In addition, wild-type DEN strains from the same serotype did not exhibit systematically the same growth profiles in these assays. In conclusion, we failed to demonstrate any correlation between attenuation or chimerisation, and replication on hepatic cells. The value of hepatic cell lines as a model for hepatotropism will be discussed.

(ACMCIP Abstract)

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LARVAL COMPETITION AFFECTS DENGUE VIRUS INFECTION IN ADULT *AEDES AEGYPTI* AND A. ALBOPICTUS

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Larval competition is well-documented among larval container mosquitoes and influences life history traits such as survivorship, development, and adult size. Few studies have attempted to address how biological interactions experienced by larvae may have carry-over effects on adult susceptibility to viral infection, subsequent viral spread to secondary tissues (i.e., disseminated infection), and viral body titer. Here we show species-specific effects of intra- and interspecific larval competition on the mosquitoes *Aedes aegypti* and *A. albopictus* comparing susceptibility to arboviral infection and dissemination using dengue-2 virus. *A. aegypti* had lower infection and body titer but higher dissemination rates than did *A. albopictus*. For both species, higher levels of intra- and interspecific competition enhanced infection and dissemination, albeit less for *A. aegypti*. Similar results have been observed for other unrelated arboviruses (e.g., Sindbis), suggesting that these conclusions may apply generally to mosquito-virus systems and that failure to consider larval competition in estimating arboviral susceptibility of vectors may result in misleading estimates of mosquito vectorial potential.

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DENGUE VIRUSES-BINDING PROTEINS FROM AEDES MOSQUITO SALIVARY GLANDS AND C6/36 CELLS

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Dengue virus (DENV), the etiological agent of Dengue Fever, is transmitted to the human host during the blood uptake of an infective vector mosquito by the injection of infectious saliva. DENV dissemination into mosquito salivary glands is therefore an essential event for the vector to become infective. In the present study, we investigated the presence of proteins able to bind all four DENV serotypes in either *Aedes aegypti* (L.) or *Aedes polynesiensis* (Marks) salivary gland extracts (SGE). We also extended our experiments to *Aedes albopictus* C6/36 cell extracts, this cell line being frequently studied as an *in vitro* model for virus/mosquito cell interactions. Using Virus Overlay Protein Binding Assay (VOPBA) we detected several DENV-binding proteins, those might be implicated in virus attachment and/or entry into mosquito C6/36 cells or salivary glands. Since this last event is necessary for the virus to be transmitted into the host, our study paves the way for the identification of target proteins that would be key elements for new "DENV transmission-blocking strategies". Otherwise, since SGE not only contain salivary gland tissue-stemmed proteins but also salivary ones, our study bring first elements for the identification of "DENV/vector salivary protein" complexes, those might interact with host immune agents at the earliest step of human infection.

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OUTBREAK OF CLASSIC DENGUE FEVER IN THE SOUTHERN PERUVIAN CITY OF PUERTO MALDONADO

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In Peru, dengue was first documented in the city of Iquitos, in 1990. Since then dengue has become endemic in many regions. In Puerto Maldonado 5 patients with positive serology for Dengue were reported in October 2001 and one dengue isolate was obtained in 2004. Here we describe the April to June 2005 dengue outbreak in Puerto Maldonado, Peru. The objective of this study was to describe the number of patients affected with dengue during April to June 2005 in Puerto Maldonado. And to describe the signs and symptoms, as well as their duration, and the age group in which the outbreak appeared. 198 patients older than 5 years were evaluated in the city of Puerto Maldonado. All patients had an acute febrile illness. Acute and convalescent phase blood samples were collected and the identification of the etiologic agents by cell culture isolation and serological assays were performed. Of the 198 patients, 51 were diagnosed with dengue: 11 by seroconversion and 40 by virus isolation. The serotype of the isolated viruses was dengue-3. The age of the patients ranged between 9 and 60 years. The average age was 29 years and 22 were males and 29 females. The signs and symptoms displayed in the acute phase were: fever in 51 patients (100%), malaise in 51 patients (100%), chills in 45 patients (88,24%), headache in 45 patients (88,24%), retro orbital pain in 43 patients (84,31%) and myalgias in 41 patients (80,39%). The remaining signs and symptoms are listed in this presentation. 44 patients were asymptomatic during the convalescent evaluation and the average of days of symptoms was 5. No patients presented symptoms compatible with hemorrhagic dengue or shock syndrome. In conclusion, this is the first confirmed outbreak of dengue fever in Puerto Maldonado. Puerto Maldonado has changed from a nonendemic to a hypoendemic dengue region of Peru.

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DENGUE VIRUS DETECTION USING AN ENZYME LINKED IMMUNOSORBENT ASSAY ON INFECTED CELL CULTURE (ELISA-ICC)

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Dengue virus belongs to the family Flaviviridae and comprises four serotypes, designated as Den 1 - Den 4. The spectrum of illness ranging from mild fever to severe hemorrhagic fever and shock. The capture ELISA test is commonly used to detect IgM antibody as indication of recent infection but the definite diagnosis of dengue is provided by the isolation/detection of virus in the patient's acute blood or sera. The common cell culture method involves visualization by IFA and most detection methods involve RT-PCR. Here we describe a novel virus identification method that combines cell culture and ELISA methodologies (ELISA-ICC). The ELISA-ICC utilizes 96 well plates in which tissue culture cells (C6/36 or Vero) have been seeded at 10^4 cells per well. Test specimen (sera/virus) is added to a well, centrifuged and incubated for 1-14 days. The cells are fixed and the presence of virus is visualized using standard ELISA methodologies. The sensitivity of detection of eight dengue viruses (Den-1: West Pac 74, IQT 6152; Den-2: S 16803, IQT 2913; Den-3: CH53489, IQT 1728; Den-4: D-4 TVP 360, OBT 1158) in C6/36, Vero and BHK cell lines was examined. The range of virus detection in C6/36 cells after six days of incubation was 0.04 - 0.0002 PFU; Vero cells: 0.04 - 2 X 10^3 PFU and BHK cells required more than 1.2 X 10^4 PFU for virus detection. Fifteen acute sera (suspected to contain dengue virus) were tested for dengue virus by standard cell culture isolation methodology, RT-PCR, ELISA-ICC(C6/36) and ELISA-ICC(Vero). Standard cell culture isolation methodology identified dengue virus in 6/15 specimens; RT-PCR: 12/15; ELISA-ICC(C6/36): 12/15 and ELISA-ICC(Vero): 11/15. In conclusion, for dengue virus detection the ELISA-ICC (C6/36) method was superior to standard cell culture isolation methodology and equal to that of RT-PCR. Advantages of the ELISA-ICC (C6/36) method over RT-PCR include a lower cost per specimen and the method is less labor intensive.

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DENGUE-2 VIRUS ALMOST ABOLISH YELLOW FEVER VIRUS REPLICATION IN C6/36 CELLS

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Yellow fever (YF) and dengue fever/dengue hemorrhagic fever (DF/DHF) are diseases caused by mosquito-borne viruses belonging to the family Flaviviridae, genus Flavivirus. Both YF and dengue viruses can be transmitted among humans in an urban cycle by the highly domesticated *Aedes aegypti* mosquito. Therefore, there is a great concern about the reurbanization of YF in Brazil due to the high prevalence of Aedes mosquitoes in this country. On the other hand, several epidemiological studies have demonstrated the absence of urban YF in Brazil as well as in several Asian countries, despite the presence of Aedes mosquitoes. Hence, one of the possible explanations that have been hypothesized to explain such phenomenon is a low Aedes mosquito's vector capacity for YF virus replication. Another explanation could be a possible cross immunity between dengue- and YF-specific antibodies. Nevertheless, none of such hypotheses have been proved yet. It was hypothesized here that *Aedes aegypti* mosquitoes infected with dengue viruses would not be permissive for YF virus infection, and that would be the reason for the absence of urban YF in highly endemic areas for both viruses. Replication profiles of dengue-2 (New Guinea C strain) and YF virus (17D strain) in C6/36 cells, a cell line derived from Aedes albopictus, were studied in co-infection assays and compared to those results obtained when cells were infected with only one virus. Viral titers were measured as either dengue-2 or YF RNA copies detected on cell supernatants by a Sybr Green reverse-transcription real-time PCR. When cells were first infected with the dengue-2 virus, and seven days later with YF virus, a reduced YF replication profile was observed while dengue-2 virus replication was kept in a high level. When cells were first infected with YF virus, and seven days later with dengue-2 virus, the replication profiles of both viruses were shown to be high-leveled. Thus, the results presented here show that dengue-2 virus interferes with YF virus replication, and since these results were obtained on mosquito cells, it is possible to speculate that once Aedes mosquitoes are infected with dengue-2 virus, a second infection by YF virus is severely impaired. Therefore, the hypothesis of dengue virus interference on YF virus replication in mosquito's cells could explain the absence of urban YF in areas where dengue is highly prevalent.

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CHARACTERIZATION OF DENGUE VIRUSES PREVALENT IN THAILAND

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Dengue virus is epidemic and endemic in virtually every country in the tropics. Dengue fever (DF) and dengue hemorrhagic fever (DHF) are serious illness in many tropical and subtropical countries. Dengue viral infection often causes viral hemorrhagic fever lead to death. A major question is why some of patients infected with dengue virus develop DHF while most of patients with symptomatic infections end up as DF. Many studies have been done in order to understand the pathogenesis of DHF. However, the mechanism of DHF remains unknown. Four types have been reported in dengue virus. Previous exposure to dengue infections increases the risk for severe diseases because antibody against dengue virus enhances the uptake of virus into macrophage. As a result of the enhancement of viral uptake, viral load is increased in patients. It possibly induces cytokine production drastically and causes DHF. Each type can be responsible for DHF. Several groups, however, reported biological differences between different types of dengue virus. All types virus have been prevalent in Thailand. However, DHF is able to occur in primary infection although it is rare. It has been reported that DEN-2 and DEN-4 can cause DHF only in secondary infection but DEN-1 and DEN-3 can cause DHF in primary infection in one fifth cases in Thailand. To understand the detail of the difference among these viruses, we characterized them by infection of C6/36 cells, HepG2 cells and J111 cells. In addition, we examined that host factors induced by infection with each dengue virus.

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HLA MAY CONTROL VIRUS SEROTYPE SPECIFIC IMMUNITY IN DENGUE INFECTION

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Dengue fever (DF) is getting a serious public health problem in the tropics. In this study, we made an experimental design to identify the host gene(s) contributing to the development of Dengue Hemorrhagic Fever (DHF), or Dengue Shock Syndrome (DSS) in Vietnamese by hospital-based case control study.

The patients with DF, DHF or DSS were clinically diagnosed by WHO criteria, and their peripheral blood samples were collected at the Center for Preventive Medicine, Vinh Long Province (VL), and the Pediatric Hospital No.2, Ho Chi Minh City (NDII) in 2002 to 2005. The patients's age ranged between 10 months and 15 years. Two hundreds age and sex matched control samples were collected in VL. The number of the patients with DF cases was 114, with DHF cases was 206, with DSS was 413 in total from two sites. HLA class I (HLA-A, B), class II (DRB1) and TNF-α promoter SNPs typing were performed.

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There was no significant difference in TNF-α promoter SNPs alleles and HLA-B. However, HLA-A*24 was significantly increase in DHF/DSS (P for trend = 0.0001) and HLA-DRB1*0901 was significantly decrease in severe patients (P for trend = 0.00011). These HLA-DRB1*0901 patients also showed resistance (P value= 0.0087) to the most virulent serotype - dengue virus serotype 2. The DRB1*0901 allele might contribute to resistance and A*24 allele might contribute to susceptible to DSS in Vietnamese. These may have consequence for preventive strategies.

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DETECTION OF DENGUE VIRAL ANTIGENS AND NEGATIVE STRAND RNA WITHIN PLATELETS SUGGESTS THE SUSCEPTIBILITY OF PLATELETS TO DENGUE VIRUS INFECTION

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The primary target cells of dengue virus (DV) infection appear to be the immune lineage cells. The majority of these cells contain nuclei. We investigate the potential for DV to infect anuclear cells, such as platelets. Platelets were isolated from EDTA-treated plasma from healthy individuals. Isolated platelets were exposed to DV for 24 hours. Lysates from platelets exposed to DV were subject to SDS-PAGE and Western blot assays using polyclonal or monoclonal antibodies. Parallel set staining with NS1 or E monoclonal antibody for FACS and confocal microscopy were performed. Anti-μ chain antibody was used as a negative antibody control and platelets exposed to media for the same periods were used as mock infected controls. Negative strand RT-PCR was performed in RNA obtained from infected platelets or from platelets in patients with dengue fever. Lysates from DV-infected HEK-293T cells were used as a positive control. Multiple DV-related protein bands, corresponding to the protein bands derived from positive control cell lysates, were observed in lysates from platelets exposed to DV. In addition, a specific NS1 protein band was seen on the membrane probed with anti-NS1 monoclonal antibody. Probing with anti-μ chain antibody did not reveal any specific proteins bands. FACS analysis showed that NS1 was expressed on the surface of DV-infected platelets. Confocal microscopy study revealed that NS1 and E proteins appeared to co-localize in infected platelets. Negative strand RNAs were detected in infected platelets and platelets isolated form patients with dengue fever, respectively. Mock control platelets were negative for Western, FACS analysis, RT-PCR, and confocal microscopy. We demonstrate that anuclear platelets are susceptible to DV infection. This finding may explain the early drop in platelet count during acute DV infections and may furthermore suggest that macrophages are secondarily infected following the engulfing of DV-infected platelets.

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USE OF THE CLUSTER INVESTIGATION METHOD FOR THE EARLY DETECTION OF DENGUE CASES: PRELIMINARY FINDINGS

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Dengue infections were first recognized in Indonesia in 1968. Since then, dengue has become endemic in many urban areas of the island chain and the frequency and severity of outbreaks have increased. In the last five years, more than ten thousands cases have been recorded annually, with most of these occurring in the capital city of Jakarta. In order to capture early cases and better understand the events that contribute to the pathogenesis of dengue infections, a community cluster investigation was initiated in the Javanese cites of Jakarta and Bandung. To date, 972 individuals from 62 communities have participated. The average interval between febrile onset and initial observation at the community averaged roughly 8 days. Dengue infection has been found in 121 (10.3%) volunteers with 45 (7.2%) from Jakarta and 76 (13.6%) from Bandung. These included 83 (7%) previous, 21 (1.8%) acute at enrollment and 17 (1.4%) acute post-enrollment dengue infections. Of the 17 acute cases, five were asymptomatic infections, 5 dengue fever cases and six dengue hemorrhagic fever (DHF) cases. Seven cases have been analyzed for primary and secondary infection. Primary infections occurred in one asymptomatic case and one DF case, whereas secondary cases occurred in one asymptomatic, 3 DF cases and 1 DHF case. Dengue viruses have been isolated and characterized from 16 of the acute cases (isolation rate 17.4%). All four dengue serotypes circulated during the study with DEN-3 (44.4%) the most predominant virus. Analysis of the association between the infecting virus, pre-illness immune status and disease severity remains on-going.

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UNDER-REPORTING OF DENGUE FEVER IN PUERTO RICO: RESULTS FROM ENHANCED DENGUE SURVEILLANCE - PATILLAS, PUERTO RICO, JUNE 2005-JANUARY 2006

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Dengue is the most common arboviral disease in the world, with 50-100 million estimated cases of dengue fever and 250,000-500,000 cases of dengue hemorrhagic fever annually. In Puerto Rico, where dengue is endemic, the Department of Health and CDC maintain a laboratory-based surveillance system in which physicians are asked to submit serum samples from suspected dengue cases for diagnostic testing. From 1996 to 2004, the incidence of reported dengue in one rural municipality, Patillas, averaged three times that of the island. Enhanced dengue surveillance was instituted in Patillas in June 2005 to better estimate dengue incidence. In Patillas (population 20,300), one clinic provides care for 87% of residents. Clinic physicians were asked to complete a case investigation form and submit serum samples for dengue testing for patients meeting a strict clinical case definition for suspected dengue: fever plus at least two symptoms (headache, eye pain, myalgia, arthralgia, rash, bleeding, thrombocytopenia, hemoconcentration, or shock). Laboratory-positive cases were defined as patients with dengue virus identified via PCR or viral isolation, seroconversion, or anti-dengue IgM positivity. From June 2005 through January 2006, the clinic reported 1096 suspected dengue cases. Of these, 136 (12%) were laboratory-positive including four hospitalized patients. The incidence of laboratory-positive dengue in this period was 6.7 per 1,000 population in Patillas, compared with 0.6 per 1,000 population for Puerto Rico and 0.1-1.6 per 1000 population for adjacent municipalities. In conclusion, the application of a strict clinical case definition in Patillas resulted in an incidence of laboratory-positive dengue infection over 11 times that of the island and at least four times that of adjacent municipalities. These findings suggest the magnitude of under-reporting of dengue fever in Puerto Rico and better illuminate the burden of dengue disease.

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DENGUE HEMORRHAGIC FEVER BY DENGUE 3 INFECTION. A RETROSPECTIVE SEROEPIDEMIOLOGIC STUDY IN HAVANA CITY, 2003

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A dengue 3 (Den3) epidemic of 12889 confirmed cases including 78 dengue hemorrhagic fever patients (DHF) including 3 fatalities was reported in Havana city in 2001-2002. This epidemic was preceded by a dengue 1 and a dengue 2 outbreaks in 1977 and 1981 respectively. According to the epidemiological dengue situation of the city, adults were at risk of a secondary or tertiary dengue infection and children at risk of their primary infection. Recognizing the uniqueness of secondary or tertiary Den-3 DHF at intervals of more than 20 years after dengue 1 (Den1) or dengue 2 (Den2) infections and understanding the opportunity to define the dengue sequences associated to the severe illness, we carried out a seroepidemiological survey in Playa municipality, one with the highest number of cases. A statistically representative sample of the population was selected using the household register of the city. Blood samples were collected from 1758 individuals and were tested by neutralization assay against the four dengue viruses. The total primary Den3 and secondary Den1/Den3, Den2/Den3 infections and tertiary Den1/Den2/Den3 infections by age for Havana city were estimated. Recent report have shown that the serological pattern of dengue infection in the 72% of patients with DHF in the city. It has been consistently demonstrated that there is a significant association of the severe disease with the sequence of infection Den1/Den3. No single case of Den2/Den3 was demonstrated associated to DHF. In addition, several individuals with a possible tertiary infection (Den1/Den2/Den3) were observed. Obtained results in both studies suggest that at least for this epidemic, the sequence of infection Den2/Den3 did not constitute a risk factor for DHF while Den1/Den3 infection did in spite that the first sequence of infection was observed in a higher frequency. DHF rate per 10,000 Den1/Den3 and per 10,000 Den1/Den2/Den3 infection were estimated. The significance of these results is discussed both in terms of the epidemiology and the pathogenesis of this disease

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THE RELATIVE TIMING OF SEASONAL WEATHER PATTERNS AND DENGUE INCIDENCE ACROSS THE SOUTHEAST ASIAN REGION

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The timing of the dengue season is often cited as corresponding with the timing of the rainy season. Here, we evaluate relationships between rainfall and other climate factors and dengue incidence data collected by passive surveillance in four Southeast Asian countries: Indonesia, Malaysia, Singapore, and Thailand; the annual timings of the rainy season and the dengue season vary widely across this region. We cross-correlate incidence time series (describing a total of 1.2 million cases) with climate data to investigate temporal relationships between changes in dengue incidence and interannual variability in rainfall, mean minimum temperature, and mean maximum temperature. Of 74 total administrative units, incidence data from 40.5% of provinces show the highest correlation with rainfall during the same month, 32.5% show the highest correlation with mean minimum temperature during the same month, and 15% show the highest correlation with mean maximum temperature two months prior. The mean high correlation between rainfall and lagged incidence time series is 0.401 (95% CI: 0.357, 0.444). Findings indicate that argued links between climate factors and changes in dengue incidence across the region remain debatable. A better understanding of the spatial and temporal dynamics of dengue infection in Asia will help investigators identify appropriate communities and seasons in which to conduct Phase III vaccine trials.

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CLINICAL AND LABORATORIAL EVALUATION OF PATIENTS WITH SUSPECTED DENGUE-3 INFECTION IN RIBEIRÃO PRETO, SÃO PAULO, BRAZIL

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Dengue is the most important arboviral disease in the world, and nowadays, dengue-3 virus constitutes a serious problem to the Brazilian public health system. Infections with dengue viruses result in different clinical syndromes, ranging from uncomplicated febrile illness to a potentially life-threatening syndrome, the dengue hemorrhagic fever/dengue shock syndrome. In this study, patients with suspected dengue-3 infections were evaluated and their clinical and laboratorial findings compared to those present in other febrile illnesses. From February 2003 to June 2003, 80 patients with suspected dengue-3 disease were submitted to a complete physical examination, and blood samples were obtained for serological testing (MAC-ELISA), virus identification by reverse-transcription polymerase chain reaction (RT-PCR), complete blood count, and measurement of liver enzymes and creatine phosphokinase. All patients were adults with ages ranging from 12 to 56 years (mean: 30.7 years). Forty-five percent were male and 55% were females. Serology results showed that 47 (59%) were IgM-positive and 33 (41%) were IgM-negative. RT-PCR detected dengue-3 genome in 20 patient samples that had been collected from 2 to 10 days of disease onset (mean: 5.7 days). All of these patients were IgM-positive. Patients' first medical evaluation ranged from 2 to 19 days after beginning of the symptoms (mean: 7.1 days). The most important findings and their frequency among IgM-positive and IgM-negative patients were as follows: thrombocytopenia (platelets <100.000/ul; 36% x 12%); AST and ALT levels 1,5-fold higher than the reference value (42,5% x 18,75% and 68% x 21%, respectively); hemoconcentration (40,4% x 31,2%); leukocytes < 4000 cels/uL (42,5% x 3%); skin rash (85,1% x 21%); positive tourniquet test (36% x 15%); itching (55,3% x 12,%); diarrhea (29,8% x 24,2%); fever (100% x 87,8%); retro-orbital pain (68% x 63,6%); headache (78,7% x 84,8%); and myalgia (89,3% x 90,9%). These results show that dengue-3 infections may be easily differentiated from other febrile illnesses, but attending physicians should not rely on "classic dengue symptoms", such as fever, headache, and retro-orbital pain. On the other hand, leukopenia, skin rash, and itching are positively associated to dengue-3 infections.

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DENGUE INCIDENCE: A TWO YEAR CONTINUED PROSPECTIVE STUDY OF DENGUE VIRUS TRANSMISSION AND DISEASE IN PRIMARY SCHOOL CHILDREN

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We report the findings of two years (2004-2005) of prospective study of dengue virus transmission and disease severity in a cohort of approximately 2010 children in 11 primary schools in northern Thailand. In 2004, 28 out of 33 confirmed (by Dengue/JE IgM/IgG EIA) acute dengue infections were positive for dengue viruses detected by nested PCR. Three out of four dengue serotypes were detected: 16 DEN-4, 9 DEN-2, and 3

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DEN-3. In 2005, 15 out of 27 confirmed acute dengue infected children were positive for dengue virus detected by nested PCR. All four dengue serotypes were detected; 15 DEN-4, 2 DEN-1, 2 DEN-2, and 1 DEN-3. Dengue serotype 4 appeared to be the dominant circulated dengue strain in Northern Thailand in 2004 and 2005. The rate of severe dengue disease in 2004 and 2005 was similar, 6/33 and 5/27, respectively. In 2004, the ratio of inapparent dengue infection to symptomatic dengue infection was 2.7:1, but ranged greatly (0.9-9.0:1). Additional data from 2005 and 2006 will be presented along with analysis on the dengue incidences, the infecting dengue serotypes, and the rate and risks for symptomatic dengue disease.

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GENOTYPING OF HONDURAN DENGUE ISOLATES BY RESTRICTION FRAGMENT LENGTH POLYMORFISM (RFLP)

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Dengue viruses exist in four antigenically distinct serotypes (DEN-1 to DEN-4), with considerable genetic variations in phylogenetically defined genotypes. In America, the reports of severe forms of dengue infections have been increasing dramatically with circulation of four dengue serotypes in endemic countries that favored the elevation of genetically diversity of these viruses. One of techniques utilized for the identification of the circulating Dengue genotypes is the Restriction Fragment Length Polymorfism (RFLP) useful to determine the origin the outbreaks, the association between infecting isolate with clinical picture and to identify virulent markers. The objective of this study was to analyze the Dengue virus serotypes circulating in Honduras by RFLP analysis, in order to determine the homology of these isolates with other viruses in the world. Seven Honduran Dengue isolates of 2003 outbreak (DEN-1, DEN-2, DEN-3) were analyzed by RFLP using the first round of RT-PCR Method Lanciotti (1992)/Rosario et al Modification (1998) Protocol. The enzymes utilized to RFLP were Hae III, Alu I, Rsa I y Hinf I (Promega Corp. USA). The RFLP analysis of 7 samples establish that the Honduran Dengue 1 strains are in close proximity with strains from Costa Rica, Brazil y Nicaragua, Dengue 2 strains are located with variants that include the strains from Cuba from the year 1997 and others from Costa Rica, Nicaragua and Brazil, with a 100% homology in the same phylogenetic branch; Dengue 3 strains are located in an independent variant from the de Cuban isolates from the epidemic of 2001-2002, but all of them with close relationship with the strains from Panama and Nicaragua. The geographic distribution of dengue viruses is rapidly expanding; in this study were concluded that all Dengue 1 strains founded are of recent circulation and all of them are related with strains in America, than have circulated between the years 70´s and 90´s; The Dengue 2 strains are related with the genotype American-Asiatic describe recently; and Dengue 3 strains have an Asiatic origin that mark the introduction of serotype 3 in America been this of particular interest. The filogenetic results obtained by RFLP are in agreement with the sequencing results reported in the literature for other countries, which can indicate that the RFLP technique can be used as a good alternative method to realize the preliminary molecular characterization of dengue isolates that are responsible of dengue outbreaks in developing countries.

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EPIDEMIOLOGICAL ANALYSIS OF JAPANESE ENCEPHALITIS IN THAILAND, 2000-2005

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The current situation of Japanese encephalitis (JE) was analyzed in Thailand from 2000 to 2005. During this period, 628 viral encephalitis cases were confirmed to be JE based on the results of anti-JEV IgM capture ELISA. JE occurred in all the 4 regions throughout Thailand, in the entire year with a small peak between April and August. The most affected age groups were 5-9 years old. and about 70% of cases were at ages from 1 to 14 years. These results indicate that JE is still a serious health problem among children in Thailand.

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DEVELOPMENT OF MURINE-HUMAN CHIMERIC ANTIBODIES FOR USE IN CALIBRATION OF SEROLOGIC TESTS FOR ARBOVIRUSES

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The current methodology for diagnosis of arboviral disease relies heavily on serological techniques such as the immunoglobulin (Ig) M antibody capture enzyme-linked immunosorbent assay (MAC-ELISA) as well as the indirect IgG ELISA, both of which require monoclonal antibodies (MAbs) specific for arboviral families. These tests are hindered by the lack of standardized calibrators and positive controls that react in assays designed for use with human sera for detection of clinically important arboviruses including dengue, West Nile, and Saint Louis encephalitis viruses. Our goal is to create chimeric antibody constructs expressing the variable regions of broadly cross-reactive murine MAbs and the constant regions of human IgM or IgG to produce positive calibrators of serologic (ELISA) assays for diagnosis of arboviral infection. The heavy and light chain variable regions from genus-specific murine MAbs for both alphaviruses (2A2C-3) and flaviviruses (6B6C-1) were amplified by reverse transcription polymerase chain reaction (RT-PCR) using degenerate primers annealing to conserved variable heavy (V_H) and variable light (V_K) gene leader sequences and constant (C) region specific primers. Multiple clones of each V_H and V_K gene fragment from 6B6C-1 and 2A2C-3 were sequenced and then modified by PCR to facilitate placement into immunoglobulin expression vectors containing genomic clones of either IgG or IgM C-region genes. A total of four separate plasmid constructs were created: pDHL-2G (2A2C-3 variable region with human γ chain, pJH-2M (2A2C-3 variable region with human μ chain), pDHL-6G (6B6C-1 variable region with human γ chain), and pJH-6M (6B6C-1 with human μ chain). These plasmids were used to transform Sp2/0-Ag14 cells by electroporation. Screening of transfectants for expression and characterization of chimeric antibodies is ongoing.

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PHYLOGENETIC ANALYSIS OF AN AVIAN ISOLATE OF WEST NILE VIRUS, LAFAYETTE PARISH, LOUISIANA (2005)

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West Nile virus was isolated from the brain of a Blue Jay (*Cyanocitta cristata*) that died in November 2005 in Lafayette Parish, Louisiana. Because very few WNV isolates from Louisiana have been genetically characterized, we compared partial nucleotide sequences (premembrane, membrane and envelope genes) to previously published strains isolated in North America and Mexico. Pairwise alignment of the Lafayette strain with homologous regions of the prototype New York 1999 strain identified the

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presence of ten divergent nucleotides. Most of the divergent nucleotides (80%) were pyrimidine-pyrimidine substitutions and only one, (U1442C) encoded for an amino acid substitution (Val-Ala at E159). A comparison of pairwise distances between all known LA isolates and NY99 demonstrates that the Lafayette 2005 isolate is the most divergent (0.5%); the overall mean distance is 0.38%. Phylogenetic analyses suggest that this isolate is part of a recently described North American clade of dominant genotypes and differs from previously sequenced Louisiana isolates. The implications of these analyses and the need for additional studies are discussed.

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PREDICTING THE TRANSMISSION OF WEST NILE VIRUS

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The intensity of West Nile virus (WNV) transmission varies enormously across both space and time, as well between species of hosts. The causes of this variability are unknown, which makes effective control difficult. We collected mosquito and bird data on WNV infection and exposure over time and space to determine drivers of WNV transmission. We found that WNV exposure varied significantly between sites, species, and over time, with some hosts experiencing nearly 100% exposure in a single transmission season at some sites and zero exposure at relatively nearby sites. We show that WNV exposure can be predicted by measuring the abundance of WNV-infected mosquitoes at a site. These data show that despite the complexity of WNV transmission it is possible to understand and predict exposure, which offers substantial promise for increasing the efficiency of control of this pathogen.

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A WEST NILE VIRUS SMALL PLAQUE VARIANT SELECTED FROM AN ISOLATE IN NEW YORK IN 2000 IS ATTENUATED *IN VIVO* AND *IN VITRO*

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A small plaque variant (SP) of West Nile virus (WNV) was selected from an isolate (WT) made from the kidney of a dead crow in New York in 2000. We have characterized SP and WT viruses *in vitro* in African Green monkey kidney (Vero), chick embryo fibroblast (DF-1) and *Aedes albopictus* (C6/36) cells maintained at different temperatures, and *in vivo* in mosquitoes, birds and mice. The SP demonstrates delayed replication and lower virus titers than the WT in Vero and DF-1 cell culture at temperatures ranging from 34° to 41°C. Significantly decreased growth of SP at 41°C in DF-1 suggests temperature sensitivity. No differences in growth rate or viral titers were noted in C6/36 cells between WT and SP at 26° to 30°C. These results suggest higher incubation temperatures may be the cause of decreased replication in Vero and DF-1. Current studies are looking at lower temperatures of incubation for these cell lines, and higher temperatures for C636. *Culex pipiens* (L.) had lower infection and dissemination rates and a higher ID_{50} following preoral infection with SP. The mean virus titers of WNV SP in mosquito bodies are significantly lower than WT. Three days postinoculation, WNV SP also had a lower rate of virus replication and lower virus loads. Chicks demonstrated delayed peak viremia and much lower virus titers in peripheral blood following infection with WNV SP compared to WT. Adult house sparrows, natural hosts of WNV, were inoculated subcutaneously with 10 e4? SP or WT. The viremic response of the SP infected birds was variable and the virus appeared to revert to WT. Further studies are underway to clarify pathogenesis in this host. C3H mice were inoculated SC in the footpad with 10^3 and 10^5 PFU WNV SP or WT. WNV SP had significantly lower morbidity and no mortality, and a lower viremia profile compared to WT. Virus was recovered with consistent high virus load from brains and foodpads of all WT mice at the time of death in one experiment and on day 7 in a second experiment, and only from foodpads of 7 of 8 infected mice following SP inoculation at 7days PI with lower virus titers. No virus was recovered from brains of SP infected mice demonstrating that SP has lower neuroinvasiveness compared to WT. All data indicate that the SP variant is attenuated *in vivo* and *in vitro.* Further studies are being conducted in mice to evaluate neurovirulence.

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PROTECTIVE IMMUNITY TO INTRATHECAL CHALLENGE OF WEST NILE VIRUS (WNV) IN A NATURALLY EXPOSED HORSE

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In an effort to understand the pathogenesis of West Nile disease in horses and the immune response to infection, a weanling, serologically naïve male horse (ID1) and a weanling, serologically positive female horse (ID2) were challenged with $5x10^5$ PFU WNV(NY crow 99 strain) via intrathecal injection with complete physical and neurological evaluations performed for 28 days post inoculation (PI). Serum and plasma samples were collected before and after challenge for serological and virological evaluation. At D28, ID1 was euthanized to further characterize the model with a full gross and histopathological evaluation performed. The other horse, ID 2 was not euthanized for further immunological studies.

Horse ID1 developed clinical signs of WN encephalitis at seven days post-infection including fever (38.4-40.1°C), depression, muscle fasciculations, ataxia, paresis, obtunded mentation, and tongue paresis. Recrudescence of signs occurred throughout the 28 day study period, clinical signs were completely resolved by D28 PI. In contrast, horse ID2 did not develop signs consistent with WNV encephalomyelitis. Mild depression occurred on D7 PI, but rectal temperature peaked at 38.7°C and no neurological deficits were noted throughout the 28 day study period. Viral cytopathic effects (CPE) were observed in Vero cell cultures incubated with EDTA plasma collected from ID1 on D1-4 PI. Evidence of viral RNA amplification in these samples was confirmed by Real Time RT-PCR. Antibody to WNV, IgM and neutralizing, was detected after infection, starting Day 10 through 28. Histopathological changes consistent with polioencephalomyelitis were observed in the brain and spinal of ID1. Neither viral CPE nor RNA was observed in Vero cell cultures and tissues, respectively. On day of infection (two months after initial screening) ID2 was negative for IgM (using MAC-ELISA), but neutralizing antibody (1:100) was detected on the serum collected before infection and throughout the study period. Cytokine analysis with Real Time PCR demonstrated the presence of IL-1β, IL-2, IL-6, IL-10 mRNA expression and limited expression of IL-4. Data obtained from this study is the first experimental evidence demonstrating immunity to rechallenge with virulent WNV in the horse and contributes to evidence that life-long immunity after flavivirus infection does occur in the horse.

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PERSISTENCE OF LYMPHATIC FILARIASIS FOLLOWING FIVE ROUNDS OF MASS DRUG ADMINISTRATION IN AN EGYPTIAN VILLAGE

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Egypt is among the first countries to have successfully completed 5 rounds of mass drug administration (MDA) with DEC/Albendazole in

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the context of a national lymphatic filariasis (LF) elimination program. The program was successful in most areas. However, surveillance data showed that microfilaremia (MF) prevalence rates remained above 1% in two sentinel sites (in 2 districts) after 5 rounds of MDA. All LF-endemic villages in these districts were treated with a 6th round of MDA. We now report data on MDA compliance and the effects of MDA on infection and transmission parameters in one of these problematic areas (Tarina village, TR). We performed annual surveys (10% randomly selected households) and assessed MDA coverage rates, MF by night blood smear, and filarial antigenemia by the ICT card test. Molecular xenomonitoring was performed with indoor resting mosquitoes pooled by household. We also assessed IgG4 antibodies to Bm14 by ELISA in primary schoolchildren (6-8 years of age) after rounds 3-5 as a marker for recent exposure to infection. Reported MDA coverage rates were 80.1%, 94.8%, 91%, 93.5% and 88.5% for MDA rounds 1-5, respectively. The MF prevalence rate decreased from 9.4% after MDA-1 to 1.5% after MDA-5 (84% reduction) whereas the antigen prevalence rate decreased from 25.1% to 8.6% (65.7% reduction). Antibody rates in grade-1 schoolchildren declined from 13.7% following MDA-3 to 4.2% after MDA-5, while mosquito infection rates declined from 8.1% (95% CI 5.85-10.47) after MDA-1 to 5.4% (3.98-7.07) after MDA-5. All of these results suggest that LF has been reduced but not eliminated in TR. This could be due to a high pre-MDA infection rate (estimated MF prevalence 15% by smear), systematic noncompliance or drug resistance (unlikely). Preliminary data following MDA-6 are not encouraging. Additional rounds of MDA would mostly treat uninfected people. Alternative options for managing areas refractory to MDA (DEC salt, vector control, targeted treatment) will be discussed.

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HELMINTH INBREEDING AND THE DETECTION AND SPREAD OF DRUG RESISTANCE

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Parasite population structure restricts helminth mate choice and will influence the spread of anthelmintic resistance. We use Wright's *F*-statistic to analyse the genotype distribution of mutations associated with selection by benzimidazole in *Wuchereria bancrofti* microfilariae. Prior to the introduction of chemotherapy the West African helminth population shows high levels of inbreeding (F_{IT} = 0.44) with high parasite genetic differentiation between the host population (increasing microfilariae homozygosity by 60%). A stochastic, individual-based microfilariae model was developed that indicates the observed homozygosity is unlikely to be solely a result of genetic sampling, demographic stochasticity, population subdivision or the sampling scheme employed. This model was used investigate the optimal sampling scheme for the detection of anthelmintic resistance. The likely implications of parasite population structure on the mass chemotherapy control programmes of human helminths presently in place and the spread of anthelmintic resistance will be discussed.

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IS IT POSSIBLE TO CONFIRM LACK OF LYMPHATIC FILARIASIS TRANSMISSION IN TOGO THROUGH NATIONAL LABORATORY SURVEILLANCE?

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To stop transmission of lymphatic filariasis (LF), WHO recommends annual mass drug administration for at least 5 consecutive years to the entire population living in areas identified as endemic before the start of the program. Evaluations in sentinel sites and spot checks are required to determine if the microfilaraemia (mf) rate is reduced to levels where further recrudescence does not occur after stopping MDA. Because LF is a focal disease, it is possible that the initial mapping or subsequent evaluations do not accurately describe the LF status of a country. After conducting mapping showing that 7 of the 35 districts were endemic, the national LF elimination program started scaling up MDAs in 2000. In 2005, sentinel and spot check sites showed that there was no demonstrable LF infection. To ensure that this represented the true epidemiologic picture, the LF program set up a national laboratory-based passive surveillance. At least one lab technician at each of the 35 district hospitals got refresher training in identification of microfilariae (mf). A system was put in place to send systematically 10 nocturnally prepared malaria thick smears from each district every month to the national coordination. Additionally, lab technicians were asked to check malaria thick smears systematically for mf and to send all positive slides to the national coordination for quality control. A decision tree for follow-up of each mf positive case includes retesting the patient, testing the community were the case is detected and the organization of MDA in case further investigation suggested that transmission was still ongoing. Preliminary results show that during the first 3 months, the national coordination received slides from 26-35 districts each month. Two slides were found mf positive, both from districts previously considered being non-endemic and an initial investigation was launched. The person identified in Bassar district could not be traced back because he was from the nomadic Peuhl tribe. The second person, a resident of Tchamba district, was identified in Blitta district. A thorough investigation will be launched and results will be reported. These preliminary results show that a passive surveillance system can be set up with minimal resources.

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COMPARING PCR AND MOSQUITO DISSECTION FOR MONITORING THE PROGRESS OF MASS DRUG ADMINISTRATION PROGRAMS FOR THE ELIMINATION OF LYMPHATIC FILARIASIS IN TANZANIA

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Delivery and coverage of mass drug administration (MDA) is vital to the effectiveness of the filariasis elimination effort. Equally important is determining when transmission has been reduced below sustainable levels. Thus, easy, effective and accurate monitoring of infection levels is critical to the elimination program. This study provides preliminary evidence of effective monitoring using mosquito infection as an indicator and aims to produce a comparison of measures across a longitudinal survey in Kirare Village, Tanzania, before and after a treatment program. The baseline pre-MDA mosquito infection rates were determined by the collection of mosquitoes during the period of Nov. 2003-Sept. 2004 by placing light traps in 50 randomly selected houses once per week. The dissection of 5,396 mosquitoes yielded a 1.4% rate of infectivity as determined by the presence of ***Wuchereria bancrofti*** L3 stage larvae. The post-MDA dissection results of 6,608 mosquitoes collected from Oct. 2004 through Sept. 2005 yielded a 0.4% infectivity rate. This 71% decrease in infectivity rate is significant with a ^{2} = 37.30, p<0.001.

In addition to monitoring by dissection, 1,514 mosquitoes collected from Jan.-July 2004 (pre-MDA) were tested in 135 pools using the molecular xenomonitoring (MX) PCR assay for *W. bancrofti*. The PCR assay estimates the mosquito infection rate by determining the presence of any stage of the parasite. Post-MDA collections of 2,374 mosquitoes collected from Jan-Oct. 2005 were tested by PCR in 201 pools. After only one round of MDA a decrease of 55% in the mosquito infection rate was found as assessed by MX, from a 15.6% probability of infection pre-MDA to a

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7% probability of infection post-MDA (estimated by Poolscreen v.2.0.2). The reduction in infection rate as shown by PCR closely parallels the decrease in infectivity determined by dissection. In addition to monitoring the progress of the treatment program in Kirare, this study is extremely important in providing comparisons and evaluations of the monitoring tools available over the course of the entire treatment period.

(ACMCIP Abstract)

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EFFECT OF THREE YEARS OF ALBENDAZOLE AND IVERMECTIN TREATMENT ON WUCHERERIA BANCROFTI TRANSMISSION IN SIKASSO DISTRICT, MALI

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Lymphatic filariasis is a disfuguring and disabling disease that represents an important public health and socio-economic problem in Mali. As part of the international campaign to eradicate lymphatic filariasis, community-based mass chemotherapy with yearly administration of albendazole and ivermectin has been initiated in the district of Sikasso. The aim of this study was to evaluate the effects of this treatment on lymphatic filariasis transmission after three yearly treatments in the village of Kolokoba in Mali (population 3551). Following baseline entomological and parasitologic surveys in 2002, yearly mass treatment of the non-pregnant population >=5 years old was instituted. The coverage rate increased over the three year period from 67% in 2002 to 72.9% in 2005. The prevalence of parasitemia, as assessed by nighttime thick smears, decreased significantly from 21.4% (n= 1141) in 2002 to 6.08% (n = 806) in 2005. The vector population was mainly composed of An. gambiae s.l. (94.62% n = 10622) followed by An. funestus. The mean man biting rate declined each year from 605 in 2001 to 276.6 in 2005. Vector infection rates also declined over time with the highest infection rate of 4.2% in 2001 and the lowest (0.2%) in 2004. Finally, the EIR (Entomological Infective Inoculation Rate) decreased by 98.99% from 13.9% in 2001 to 0.14% in 2005. Although complete interruption of transmission has not occurred after three years of mass treatment in this area, significant decreases in both the prevalence of parasitemia and annual transmission potential have been observed. Increasing coverage rates and treatment of surrounding villages as the program expands will likely lead to improved results in the coming years.

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MONITORING OF *WUCHERERIA BANCROFTI* PARASITISM IN AN ENDEMIC SENTINEL SITE: A THREE TIME-SURVEY OF ADULT WORM INFECTION LEVEL IN HUMAN POPULATION AND LARVAE CIRCULATION THROUGH *AEDES POLYNESIENSIS* MOSQUITO-VECTOR

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We studied and evaluated the evolution of *Wuchereria bancrofti* parasitism in the whole population (near 1 400 people) of a filariasis-endemic area in Raiatea Island, French Polynesia, all along a 6 year period with annual DEC-ALB mass drug administration (MDA). Repeated surveys (pre-MDA evaluation in 2000, intermediate evaluation in 2003 and final evaluation in 2006) included tests for adult worm circulating antigens, microfilariae and anti-filarial IgG in human blood as well as PCR-based poolscreening estimation of *Ae. polynesiensis* mosquito infection. Global results show that despite a MDA compliance rate between 79% and 88%, the evolution of the studied parameters is rather limited. Transmission indices represented by prevalence of microfilariae carriers and mean infection rate in mosquitoes only slightly decreased respectively from 7% to 4% and 2.5%[2-4] to 0.8% [0.3-2]. Besides, parasitism index constituted by the prevalence of people positive in adult worm circulating antigens were shown to maintain above 10%. A detailed analysis of the area divided into 4 geographical sections is presented with the aim to scan such a situation and try to point out determinant factors.

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EFFECTS OF NITAZOXANIDE, TIZOXANIDE, AND NITAZOXANIDE WITH DIETHYLCARBAMAZINE ON THE FILARIAL NEMATODE *BRUGIA MALAYI* IN VITRO

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Lymphatic filariasis caused by *Wuchereria bancrofti* and *Brugia malayi* (Bm) is a major cause of disability in the developing world. Currently recommended treatments (albendazole with diethylcarbamazine or ivermectin) are most effective against microfilariae (MF) and less active against adult worms. Therefore, there is an urgent need for improved treatment of filarial infections that can safely and effectively kill adult filarial worms. Prior studies have shown that the antibiotic nitazoxanide (NTZ) exhibits broad activity against anaerobic bacteria, protozoa, and certain intestinal helminths. The purpose of this study was to examine the effects of NTZ and its active metabolite tizoxanide (TZ) on Bm adult worms and MF *in vitro*. Adult worms were cultured *in vitro* with NTZ or TZ at 37 C in RPMI-1640 medium with 10% FCS for 8 days. MF were cultured with or without NTZ and TZ for 72 hr. NTZ and TZ reduced worm motility and viability (assessed by MTT reduction) in a dose-dependent manner. Worm viability was reduced by 50% with both compounds at 2.5 µg/ml. 20 µg/ml killed all adult worms. 5 µg/ml of NTZ and TZ reduced MF release by 50% after 2 days in culture. Embryograms showed that NTZ and TZ blocked embryogenesis at an early stage. MF motility was also decreased by these drugs, and 10 µg/ml killed 50% of MF by 72 hr. Scanning EM showed damage to the adult worm cuticle at the anterior end of treated worms. Transmission EM revealed abnormal mitochondria in treated worms with no apparent changes in hypodermis or muscle. *Wolbachia* DNA levels in adult worms were not significantly affected by treatment. These findings suggest that NTZ and TZ may act by interfering with anaerobic electron transport. NTZ also exhibited synergy with diethycarbamazine (DEC) for reducing MF release and killing adult worms. In summary, our results show that NTZ and TZ have potent effects on Bm adult worms and MF *in vitro*. DEC enhances the activity of low concentrations of NTZ.

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ROLE OF GLICOCORTICOIDS IN THE INNATE AND ACQUIRED IMMUNE RESPONSE OF MICE INFECTED WITH *STRONGYLOIDES VENEZUELENSIS*

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Dexamethasone (Dexa) presents anti-inflammatory action and is used worldwide in urticaria, allergy and asthma treatment. When human patients with chronic strongyloidiasis are submitted to treatment with this drug, they develop a disseminated form of infection by such parasite. This study aimed at investigating the role of Dexa in the increase of eosinophils in the blood and the recruitment of these cells into the peritoneal cavity fluid (PCF) and bronchoalveolar space (BALF) in mice infected with *S. venezuelensis.* BALB/c male mice weighing 16 to 20 g were s.c. infected with 1500 infective larvae of *S. venezuelensis* and daily treated or not with 2 mg/kg of Dexa s.c according to three treatment schemes: 1. The animals were treated for seven days and infected on the 7th day; 2. The mice received the first dose of Dexa one hour prior to infection and the

last dose one hour before they were sacrificed; 3. The animals received the first dose on the 4th day after infection. Non-infected mice were used as control. On the 1st, 3rd, 5th, 7th, 14th, 21st and 37th day after infection, the animals were killed and the number of eosinophils in the blood, PCF and BALF as well as cytokines, antibodies and the number of larvae and worm parasites were quantified. Eosinophil number increased significantly in infected mice when compared with the control group. Dexa significantly inhibited the number of eosinophils in the blood as well as migration to PCF and BALF in the three treatment schemes. Dexa decreased the synthesis of TNF-α, IL-3, IL-4, IL-5, IL-10, IL-12, IFN-γ and IgG1, IgG2a and IgE antibodies. However, the number of larvae and recovered worm parasites were significantly increased. In conclusion, our data showed that Dexa is a potent immunosuppressive drug which is capable of inhibiting eosinophilia, cytokines and antibodies as well as helping parasitism in this experimental model.

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VARIATION IN PREVALENCE OF PARASITES AS A FUNCTION OF ALTITUDE IN BOLIVIA

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A parasitological survey was carried out in 15 localities in Bolivia, with altitudes ranging from 375 m to 2,125 m associated with great variation in climate and geology. Blood and faecal samples were obtained from 2,132 and 1,855 people respectively. Haematocrit was measured in the 2,132 blood samples and titration of ***Trypanosoma cruzi*** antibodies was carried out only in a target population (children under 3 and their mothers, i.e. 304 people). Faecal samples were analysed by MIF. Possible risk factors considered were (1) age, (2) gender, (3) family structure, (4) family size, (5) house size, (6) quality of house construction, (7) access to water, (8) place used for defecation, (9) economic indicators (e.g. quality of household appliances, number of cattle or poultry), and (10) length of residence in the village.

A highly significant positive correlation ($P < 10^{-3}$) was found between altitude and either haematocrit or prevalence of Chagas disease. A highly significant negative correlation ($P < 10^{-3}$) was found between altitude and prevalence of either anguillulosis or ancylostomosis. A weaker but still significant ($P < 5{\cdot}10^{-2}$) negative correlation was observed between altitude and prevalence of trichocephaliasis.

A highly significant positive correlation ($P < 10^{-3}$) was found between presence of anguillulosis or ancylostomosis and family size. A highly significant negative correlation ($P < 10^{-3}$) was found between quality of house construction or house size and prevalence of either anguillulosis or ancylostomosis. A weaker but still significant ($P < 5{\cdot}10^{-2}$) negative correlation was observed between age and prevalence of ancylostomosis, between length of residence in the village and prevalence of either anguillulosis or ancylostomosis and between poultry and prevalence of Chagas disease and amoebiasis. A weaker but still significant ($P < 5{\cdot}10^{-2}$) positive correlation was observed between access to water and prevalence of amoebiasis, presence of mammals near the house and prevalence of ancylostomosis.

Chagas disease is strongly correlated with altitude. The most significant risk factors for presence of anguillulosis and ancylostomosis are (1) low altitude, (2) large family size, (3) small house size, and (4) house construction using poor materials.

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PREVALENCE AND INTENSITY OF GEOHELMINTH INFECTIONS IN SCHOOL-AGE CHILDREN FROM THE IZABAL REGION, GUATEMALA

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Geohelminth infections are a leading cause of chronic anemia and malnutrition worldwide, with a particularly high impact in impoverished rural communities. School-based deworming programs have been demonstrated to improve the growth and nutritional status of children living in areas endemic for geohelminths, including *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm. As part of a study aimed at defining the optimal deworming regimen in high risk populations, children from 10 primary schools in the Department of Izabal in eastern Guatemala were assigned to receive single dose albendazole treatment at either 6-month or 3-month intervals. Baseline enrollment data included analyses of 344 children aged 5-17 years. Of those, 229 stool samples were examined for the presence of helminth eggs via direct microscopy and the McMaster technique. Samples positive by either method were subsequently evaluated using the Kato-Katz technique in order to measure the intensity of infection (eggs per gram of feces). Of the 229 children examined, 69% were infected with at least one geohelminth: 30% for hookworm; 52% for *Ascaris*; and 38% for *Trichuris*. Among infected subjects, 59% had infection with 2 or more geohelminth species. Infections were more common in males than females (54% vs 46%), and more common in children living in rural than urban areas (56% vs. 44%), but neither difference was statistically significant. Anemia (blood hemoglobin level <11g/dL) was present in 4% of the 297 children studied, and occurred more commonly in geohelminth infected than uninfected subjects (13.1 g/dL vs 13.5 g/dL, p= 0.05), and in those living in close proximity to a lake (12.7 g/dL vs 13.3 g/dL, p= 0.0004). Based on infection intensity thresholds defined by the WHO, only 2% of hookworm infections were heavy, as were 4% of the *Ascaris* infections and 1% of the *Trichuris* infections. Increased intensity of hookworm infection was associated with a decrease in blood hemoglobin levels (p = 0.004), while no similar association was noted with *Ascaris* or *Trichuris.* These data confirm a high pre-treatment prevalence of geohelminth infections in school-aged children in eastern Guatemala. Future analysis of post-treatment growth and nutritional data will help define the optimal dosing regimen for children in this highly endemic area.

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PECULIARITIES OF ANCYLOSTOMA CEYLANICUM L3 EXTRACTION

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The Gates Foundation sponsored hookworm project, resulting in the first generation of human hookworm vaccine, maintains three hookworm strains on experimental animals, two of them - *Ancylostoma ceylanicum* and *Necator americanus* on golden hamsters. These hookworms have quite different infective power for golden hamsters and egg concentration in feces of infected animal. The appearance of their eggs under microscope is also different. *A. ceylanicum* infective L3 larvae are more vigorously mobile than those of *N. americanus*. Contrary to *N. americanus* L3, *A. ceylanicum* L3 preserve their mobility longuer in deionized water (DD) than in tap water (TW). As the basis for L3 coproculture extraction is larval movements between charcoal granules and thru Kimwipes lab tissue (Kimberly-Clark), we hypothesized that extraction in deionized water could increase the L3 yield. This hypothesis was tested by parallel extraction of coproculture aliquots in DD and TW. In all our experiments

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the L3 yields in DD were higher than in TP, mean of DD/TP ratios is 125%. Another A. ceylanicum extraction peculiarity is at level of the ratio second (overnight) extraction versus first (3 hours) extraction. This ratio is 64% for *A. ceylanicum* (mean of 7 experiments) but for N. americanus 6% (means of 10 experiments) and for *N. americanus* coproculture under incubation for over 10 days only 2%. Perhaps the size of these hookworm infective larvae could explain the last peculiarity.

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HEMOZOIN DIFFERENTIALLY AFFECTS HIV-1 VIRAL REPLICATION ACCORDING TO THE SEQUENCE OF EXPOSURE

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Malaria and HIV-1 infections are responsible for 18% and 6%, respectively, of the mortality in African children under the age of five. Although co-endemicity of these pathogens is common in many tropical regions, molecular interactions between malaria and HIV-1 are largely undefined. Previous studies in our laboratory demonstrated that exposure to malarial pigment (β-hematin, synthetic hemozoin, sHz) increases simian immunodeficiency virus (SIV) production in rhesus macaques through a TNF-α-dependent mechanism. To further define interactions between malaria parasite products and immunodeficiency virus replication, we determined if the sequence of sHz exposure alters HIV-1 viral replication. This was accomplished by alternating the initial exposure of PBMCs with either sHz or HIV-1. sHz treatment of PBMCs from healthy donors five days prior to HIV-1 infection (monotropic HIV-1BAL) decreased HIV-1 p24 production, while sHz-treatment following 24hrs of HIV-1 infection increased HIV-1 p24 concentrations in culture supernatants relative to controls. In addition, an intracellular p24 assay was used to determine the amount of virus entering the cells. PBMCs were treated with sHz five days prior to a 3hr incubation with HIV-1BAL, then washed extensively to remove free virus, lysed, and the supernatants assayed for p24. These experiments revealed that treatment with sHz prior to HIV-1 exposure decreased HIV-1 particle entry. Cell viability was appropriately controlled for in all experimental paradigms with a tetrazolium salt-based assay. Taken together, these studies demonstrate that the effect of sHz on HIV-1 viral replication is markedly different depending on the sequence of exposure to the two pathogens. Since our previous studies showed that sHz differentially regulates β-chemokine gene expression in cultured PBMCs, differential effects presented here may be related to chemokines competing for receptor binding with HIV-1. Future studies will determine the influence of chemokine/chemokine receptor interactions on HIV-1 viral replication during malaria/HIV-1 co-infection.

(ACMCIP Abstract)

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TOXICITY OF NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS (NRTI) IN MICE

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It is difficult to evaluate the toxicity of dual NRTI therapy in infants prenatally exposed to dual NRTI therapy. Thus, appropriate animal models are needed for testing and selecting safer combinations of antiretrovirals in order to prevent embryonic tissue damage. In this study, we exposed mice to NRTIs chronically, at doses calculated to cause limited toxic reactions in the dams but sufficient damage in the pups. We administered AZT and 3TC orally to 50 CD-1 pregnant mice divided into 3 groups according to treatment doses. We gave AZT and 3TC from E8-9 till birth D1-2 by oral gavage, at a dose of 0 (control), 0.3/0.15 mg (treatment 1), 1.2/0.6 mg (treatment 2), and 4.8/2.4 mg (treatment 3). Tissues from liver, spleen and bone marrow were collected immediately after birth. The number and percentage of monocytes, lymphocytes and neutrophils were computed using standard techniques. We also evaluated the expression of CD3 and CD45 in the liver and spleen by immunohistochemical procedures. We used ANOVA to compare the differences in treatment responses. We considered a p-value < 0.05 as statistically significant. We found an inverse correlation between antiretroviral dose and CD3/CD45 expression in the liver and spleen of mothers and pups. CD3 expression was also significantly reduced in brain tissues. The mean lymphocyte value for group 2 (34) and group (16.5) was significantly lower than for the control group (41.6); the mean macrophage value for group 2(6) and group 3 (5.05) was significantly lower than for group 1 (9); and the mean neutrophil value for group 2 (6) and group 3 (5.05) was significantly lower than for group 1(9). In conclusion, these results seem to suggest that AZT and 3TC induce lymphocyte toxicity. To determine the mechanisms of lymphocyte depletion, we need to conduct more studies including further observation of the mitochondria and related organelle changes. Long-term implications of large-scale perinatal therapy around the world are still poorly understood. These issues certainly underscore the need for animal model studies, which serve to select a safer perinatal antiretroviral drug regimen.

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VALIDATION OF A LOW-COST SYSTEM FOR CD4+ T LYMPHOCYTE ENUMERATION IN RURAL BURKINA FASO

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Community-driven development (CDD), a model where resources are put in the hands of the beneficiary communities, has now been applied to the model of Association Driven HIV/AIDS Care and Treatment (ADCT) in several developing countries. Given the high prevalence of HIV disease, an accurate, low-cost method for CD4+ T lymphocyte monitoring to stage HIV infection and guide antiretroviral therapy is urgently needed. The EasyCD4 system (Guava Technologies, Inc.) uses microcapillary flow cytometry and has several advantages in comparison to standard technology. The study objective was to compare the performance of the EasyCD4 system to the FACSCount (Becton, Dickinson and Company) for community-based CD4+ monitoring of patients with HIV/AIDS in rural Sub-Saharan Africa. Blood samples from 98 HIV positive patients followed in a community HIV clinic in Ouahigouya, Burkina Faso were obtained for routine CD4 count monitoring. Each blood sample was divided into two aliquots, on which parallel CD4 count measurement was performed using the EasyCD4 and the FACSCount systems. Spearman rank correlation coefficient was calculated. Sensitivity, specificity and positive predictive value (PPV) for EasyCD4 <200/mm3 were determined compared to the standard FACSCount CD4<200/mm3. Sensitivity, specificity and PPV were calculated using the breakpoint EasyCD4 <243.5 based on classification and regression trees. Mean CD4 count for the EasyCD4 and FACSCount were 313.75 and 303.47, respectively. Correlation coefficient (*r*) using the two methods was 0.92 (p<0.001). Median values using EasyCD4 were higher than those with the FACSCount (p=0.004). Using EasyCD4 <200, sensitivity is 71.8% (CI 55.1-85%), specificity is 94.9% (CI 85.8-98.9%), and PPV is 90.3% (CI 74.2-98%). Using EasyCD4 <243.5, sensitivity is 92.3% (CI 79.1-98.4%), specificity is 88.1% (CI 77.1-95.1%) and PPV is 83.7% (CI 69.3-93.2%). These results demonstrate that use of the EasyCD4 system is feasible for the enumeration of CD4+ T lymphocytes in rural Sub-Saharan Africa, particularly in the context of community-based HIV/AIDS healthcare. Additional studies will be required to determine the appropriate thresholds for initiation of antiretroviral therapy using low-cost diagnostic technology.

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HIV/VL CO-INFECTION: AN INDIAN EXPERIENCE WITH SPECIAL FOCUS IN BIHAR

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HIV Syndrome is caused by human retrovirus (Retroviridae), the subfamily of lentivirus. The current estimate of cases of HIV infection among adults worldwide is nearly 37 million, two-thirds of whom are in sub Saharan Africa and 50% are women. Visceral leishmaniasis, an insect transmitted disease, is one of the most neglected diseases, transmitted by the bite of infected female phlebotomine sandflies and the causative agent is a protozoan *Leishmania donovani*. The disease is prevalent worldwide and the estimated global prevalence and incidence per year is 2.5 and 0.5 million respectively. Bihar, an eastern Indian state, contributes to about 80% of the total Indian cases. The simultaneous prevalence of HIV and VL in the same endemic regions and immuno-suppression by both the diseases influence the increasing HIV-VL co-infection cases worldwide. The co-infection of HIV-VL is also rising in India and is assuming dangerous proportions. In context of Bihar, it has been observed that daily wage earners from the low socio-economic group of VL endemic villages travel to economically advanced, but also high prevalence zone for HIV, places like Mumbai, Chennai, Kolkata etc for their bread and butter. It is assumed that these classes of people contract the HIV by indulging in high- risk sexual behaviour and after returning to their native villages, they infect their wives and thereby they spread the HIV infection in their family. About 30-45% of HIV-VL co-infected patients do not show all the VL suggestive clinical manifestation, 10-15% show only fever with gastro-intestinal involvement, weight loss and pulomonary infection mainly tuberculosis or Pneumocystis carinii pneumonia (PCP). About 10% do not show any manifestations of VL but they remain positive parasitologically. Treatment of HIV/ AIDS infection with VL and other opportunistic infections like tuberculosis, CMV, PCP etc. makes the therapy too costly to be afforded by the middle class people and beyond the means of the poor masses.

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EFFECTS OF PNEUMOCOCCAL CONJUGATE VACCINE (CV) FOLLOWED BY PNEUMOCOCCAL POLYSACCHARIDE VACCINE (PV) ON THE TYPE-SPECIFIC IGG LEVELS AMONG HIV-INFECTED ADULTS IN UGANDA.

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HIV-infected Africans are susceptible to pneumococcal infections. The effects of pneumococcal polysaccharide vaccine (PV) in HIV remains controversial. Furthermore, a previous study in Uganda showed that PV could provide an increased risk of invasive infections and no protection in HIV-infected patients. An enhanced antibody response to PV after prior immunization with CV has been shown in a previous study. This study was, therefore, conducted in order to determine the effect of immunization with CV followed by PV on the serum levels of type-specific IgG in HIV-infected adults with CD4 >200/Î1/4l in Uganda. After screening for HIV-status and CD4 counts, HIV-uninfected and infected healthy subjects were enrolled at Joint Clinical Research Centre, Kampala, Uganda. Subjects were classified into three groups: Group A; HIV(+) CD4:200-500/ Î**1/4**l (mean age; 38 y.o.; mean CD4: 352), Group B; HIV(+) CD4> 500/ Î**1/4**l (mean age; 37.1 y.o.; mean CD4: 720), Group C; HIV (-) (mean age; 26.8 y.o.; mean CD4; 882). These subjects were immunized with 7-valent CV followed by 23 valent PV. The levels of type 4 and type 14 specific IgG in sera were determined before and 1 month after CV, and 2 months after PV by 3 rd generation ELISA. Before vaccination, the levels of type 4 specific IgG were higher in group A than in group B or C, and the levels of type 14 were similar among three groups. Immunization with CV significantly increased the levels of type 4- or type 14- specific IgG among three groups. While the levels of type 4 specific IgG were higher in the group C than in Group A or B, the levels of type 14-specific IgG were higher in group C> group B> group A. The second-dose with PV produced no further increase in type-specific IgG levels. Our data suggest a promising serological effect of CV in induction of type-specific IgG among HIV-infected adults with CD4 higher than 200/Î¼l. PV after immunization with CV appears to be not useful. A single dose of CV could be a new strategy against pneumococcal infections in HIV-infected adults before introduction of antiretroviral therapy in HIV-endemic, developing countries.

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IMPROVED DIAGNOSIS OF CRYPTOSPORIDIAL AND MICROSPORIDIAL INFECTIONS BY PCR IN PATIENTS WITH AIDS AND DIARRHEA IN HAITI

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Enteric parasitic infections commonly cause diarrhea in patients with AIDS in resource-limited settings. They result in decreased quality of life and may compromise antiretroviral therapy (ART). Sensitive diagnostic tests to diagnose these potentially treatable infections are often unavailable in these settings. An on-going study at the GHESKIO Centers in Port au Prince, Haiti is examining patients with diarrhea at the initiation of ART. From October 2005 to date, 25 patients with diarrhea have been enrolled. 23 stool samples, collected at enrollment, are available for analysis. At GHESKIO, standard practice for stool evaluation includes a wet mount for ova and parasites and a modified Kinyoun acid-fast stain for coccidial parasites. We used real-time PCR (qPCR) to detect the presence of *Cryptosporidium* DNA and to quantify oocysts from the stool samples. We also used a PCR assay that amplifies a conserved region of the small-subunit rRNA of all four major microsporidian pathogens, *Encephalitozoon cuniculi*, *Encephalitozoon hellem*, *Enterocytozoon bieneusi*, and *Encephalitozoon intestinalis,* followed by restriction endonuclease digestion by *PstI* to determine the prevalence of each species in the stool samples. 10/23 patients (43%) had stools positive for *Cryptosporidium* DNA using qPCR. The median number of *Cryptosporidium* oocysts for patients was 700 per ml of stool. In contrast, only 3/23 (13%) were positive for *Cryptosporidium* by acid-fast staining. Restriction analysis indicated *E. bieneusi* DNA in 4/23(17%) patients. No Microsporidia were identified by microscopy. The sensitivity of the current practice for the identification of *Cryptosporidium* infection in Haitian AIDS patients with diarrhea is 30%. In colclusion, molecular diagnostics for *Cryptosporidium* and *E. bieneusi* in AIDS patients with diarrhea improve rates of diagnosis of these potentially treatable infections. As nitazoxanide comes into wider use, accurate diagnosis of cryptosporidial infection may become more important. Albendazole can be used to treat Microsporidia infection. Continued efforts to develop rapid molecular diagnostic tests for resource-limited settings are essential.

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ORAL MILTEFOSINE FOR CUTANEOUS LEISHMANIASIS IN THE DUTCH ELECTION SUPPORT FORCE IN NORTHERN AFGHANISTAN

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Preliminary results of treatment with oral miltefosine for cutaneous leishmaniasis in Dutch military personnel and one civilian who contracted cutaneous leishmaniasis in Northern Afghanistan in 2005 are presented. Initial therapy in 175 patients was with intralesional sodiumstibogluconate, in some patients combined with cryotherapy. Thirtythree non-responders to topical therapy and one therapy naïve patient were treated with miltefosine. The diagnosis was confirmed parasitologically in all 34 patients at initial presentation. *Leishmania major* was demonstrated in all PCR-positive cases. Treatment with miltefosine was 50 mg 3 times a day for 28 days. Patients' body weight ranged from 70 to 112 kg. Response to treatment was assessed clinically and by parasitological methods of PCR and Quantitative Nucleic Acid Based Amplification (QtNASBA). Assessment was done at the end of treatment, at 6 and 24 weeks thereafter. Nausea was reported by 21 (61.8%), vomiting by 14 (41.2%) and fatigue by 10 (29.4%). Five patients (15.3%) spontaneously reported a transient reduction of seminal fluid volume during treatment. Two of these patients also reported transient scrotal pain and epididymal swelling. Mild elevation of transaminases and creatinine was seen in 6 (17.6%) and 1 (2.9%) respectively. All abnormalities were reversible. Adverse effects did not lead to non-compliance or absenteeism. 29/34 (85.3%) patients presented with lymphonodular involvement during treatment. This lymphonodular reaction was also seen in untreated and topically treated *L. major* infections. Follow-up is on-going. Directly following treatment, 19/34 (55.9%) and 16/30 (53.5%) showed clinical and parasitological recovery by PCR, respectively. After 6 weeks 21/31 (67.7%) and 21/29 (72.4%) showed clinical and parasitological recovery by PCR, respectively. Three patients (8.8%) have shown recrudescence requiring additional treatment. In conclusion, miltefosine is a reasonably well tolerated and effective treatment for cutaneous leishmaniasis due to *L. major* in Northern Afghanistan.

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PARASITOLOGICAL AND HISTOLOGICAL STUDIES IN SKIN FROM DOGS NATURALLY INFECTED WITH LEISHMANIA (L.) CHAGASI IN ILHA SOLTEIRA, SP, BRAZIL

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Canine visceral leishmaniasis is caused by a protozoal parasite of the specie Leishmania (L.) chagasi, endemic for humans and dogs in many regions of Brazil. The aim of this study was to investigate the parasitological and histological pattern of the skin lesions from 34 dogs grouped in symptomatic (n = 9), oligosymptomatic (n = 17) and asymptomatic (n = 8) according to the clinical signs of the disease. All dogs were submitted to direct parasitological examination by direct microscopic visualization of promastigotes in stained tissue smears or in paraffin embedded tissues (histopathology) and serological examinations (ELISA and IFAT). The dermal inflammatory pattern and the cell population were evaluated histologically on HE-stained sections. The parasite load was determined by 100 microscopic field examination (magnification of 1000x) and it was graded from + to ++++. In the asymptomatic group: 4 dogs (50%) were negatives and 4 were positives showing grades ranging from + (12%), ++ (25%) to ++++ (12%). The oligosymptomatic group had 2 (12%) negatives and 15 infected with grade from + (29%), ++ (35%), +++ (12%) to ++++ (12%). The asymptomatic group had only positive dogs with the grade ranging from + (22%), +++ (11%) to ++++ (67%). The majority of the parasites were loaded inside the macrophages near to the epidermis or in the midi-dermis around the small vessels, hair follicles and sebaceous glands. The most severe lesions and the greatest parasite load were seen in the symptomatic dogs. The most of tissues showed areas with chronic inflammatory lesion characterized by the presence of large numbers of macrophages, lymphocytes and plasma cells. 92% of skin with microscopical lesion and only 40% without lesion were positive with amastigotes. The serological tests showed that 62% of asymptomatic dogs, 18% of oligosymptomatic and 12% of symptomatic were negatives and there was no agreement between serological and parasitological examination in 9% of the cases.

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FIELD STUDY OF A NOVEL MULTIPLE ANTIGEN BINDING ASSAY (MABA) FOR THE DIAGNOSIS OF LATENT CHAGAS DISEASE IN AN ENDEMIC COUNTRY

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There is no gold-standard test for the diagnosis of latent Chagas disease. Multiple antigen binding assay (MABA) is attractive because it can be simple, rapid and specific. In the MABA format, different antigens can be used on the same strip, conserving serum. The objective of this work was to assess a MABA test based on trypomastigote excreted/secreted antigen (TESA Tulahuen) and a 85 kDa immunoaffinity purified TESA protein as a confirmatory test for latent Chagas disease. A total of 66 individuals were recruited: 34 female (52%) and 32 male (48%) and mean age 23 years (standard deviation: 14.17), in Rio Brito (Sucre state, Venezuela), a rural community with heavy domestic triatomine insect infestations rates. Serum samples were analysed by an EIA using fixed-epimastigote antigens that were grown in axenic culture (LIT medium) and the novel MABA test. The EIA test identified 7 positive sera (detection rate 11%) only 6 of which were confirmed by MABA (detection rate 9%). These data suggested that MABA test could be used either as a second 'routine' test (eg: for blood bank screening) or as a confirmatory assay for the diagnosis of latent Chagas disease. The high detection rate for anti-*Trypansoma cruzi* antibodies found suggest a clear lack of an adequate control of Chagas disease in this region of Venezuela.

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GEOGRAPHICALLY ROBUST LATERAL FLOW IMMUNOASSAY FOR DIAGNOSIS OF *T.CRUZI* INFECTION WITH HIGH CORRELATION TO RADIO-IMMUNOPRECIPITATION ASSAY (RIPA)

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The incidence of seropositive blood donors in North America has increased with population migration and increased surveillance. The USA, considered non-endemic for *Trypanosoma cruzi,* could therefore be at risk to exposure to disease transmission through blood or organ donations. Current tests show variable reactivity, especially with Central American sera. Here we describe the development of a lateral flow immunoassay for rapid detection of *T.cruzi* infection that has a strong correlation to the RIPA "gold standard" and is applicable for use in geographically diverse samples particularly from Central America. Such a test would have utility in small blood banks for pre-screening donors as well as in cardiac transplantation evaluation. *T. cruzi* consensus and/or RIPA positive sera were evaluated in EIA. These included commercial panels from Boston Biomedica Inc (BBI) (n=14), and Teragenix (n=21). Both sources represented samples from different Central and South American countries. Other sources included a blood donor panel mainly from Costa Rica, Nicaragua, Honduras and Guatemala (n= 205) as well as RIPA positive sera from the American Red Cross (ARC) (n=44). Sera were tested with the multiepitope recombinant TcF and Peptides 1,30,36,SAPA and 1.1,1.2 and 1.3His. Of the Central American blood donor panel tested only with TcF, 31 were positive. 29 were positive by Organon EIA and 172 negative by both tests. 2 were Organon EIA positive only. In addition, all BBI samples were positive and 7/21 Teragenex samples and 6/44 ARC samples were low positive or negative. This indicated the need for additional antigens. To complement TcF reactivity in Central American sera we identified a promising combination of the tested antigens and constructed a single recombinant protein that exhibited full reactivity of the complementary antigens and enhanced clinical sensitivity in Central American sera. Data on its evaluation with RIPA confirmed positive sera will be presented. In addition, antigens identified from serological expression cloning with RIPA positive, TcF negative sera are being evaluated for potential use in the rapid assay as necessary.

(ACMCIP Abstract)

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PREVALENCE OF TRICHOMONIASIS IN IMO STATE, NIGERIA

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Trichomoniasis caused by the protozoan parasite, *Trichomonas vaginalis,* is a common sexually transmitted disease(STD) all over the world. But it is most *prevalent* in third world countries. The importance of *T. vaginalis* as a sexually transmitted disease agent cannot be over-emphasized. Apart from its known pathological effects, it is a risk factor for HIV/AIDS. The purpose of this study was to find out the trend of trichomoniasis in Imo State, Nigeria. Between June 2004 and September 2005, test samples were collected from both male and female respondents ranging in age from 11 to 51 years and above and examined for *T. vaginalis* infection. Of 8,439 specimens examined, 241(2.86%) were positive. Based on previous studies conducted, the prevalence of trichomoniasis in Imo State appears to be abating.

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EPIDEMIOLOGICAL AND CLINICAL PATTERN OF CUTANEOUS LEISHMANIASIS FROM A REFERRAL HOSPITAL IN MALI

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Cutaneous leishmaniasis (CL) was first reported in the 1960s in several areas of Mali with 2 principal foci identified in the regions of Kayes and Segou located in the western and the mid eastern part of the country, respectively. It is not known whether any changes have occurred in the epidemiology and clinical patterns of the disease since these early reports. Yet, the parasites responsible for the disease in the country need to be specified. The study was undertaken to describe the clinical and geographical distribution of CL and to determine the parasite responsible for the disease in Mali. Between January 2005 and April 2006 CL cases were recorded at the Dermatologic Institute of Bamako, the sole referral dermatologic clinic. Biopsy was taken from the lesion edge and smeared onto a slide. The slide was dried, fixed with methanol, Giemsa-stained, and examined under the microscope for presence of *Leishmania* amastigotes. The diagnosis of CL was based on confirmation of clinically suspected cases by microscopy. The parasite identification was made by PCR. A total of 95 clinically and confirmed cases of CL were recorded. The mean age of patients was 32 years old (varying between 14 month and 70 years). Although patients were received from all over the Sahelian and north Sudan savanna areas of Mali, 43% and 28% of cases were residents of Kayes and Koulikoro region, respectively, in central Mali. The maximum number of cases was recorded in between January and February (32%) during the dry season. The ulcero-crusted form (75.8%) was the most frequent clinical form observed. However 30% of cases were superinfected with microbial and fungal infection. Atypical form including disseminated cases associated with HIV infection was also observed. PCR performed on 41 specimens showed that L. major is the parasite responsible for CL in Mali. In conclusion, this study shows that CL remains prevalent in Sudan savanna and Sahelian areas of Mali. Clinical feature is characterized by microbial surperinfection. An increase of the awareness of clinicians on CL at peripheral health district is needed to establish a country-wide surveillance system.

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INCREASED VERTICAL TRANSMISSION OF A NORTH AMERICAN TYPE II ISOLATE OF *T. CRUZI* AS COMPARED TO THE TYPE I BRAZIL STRAIN

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Vertical transmission of *Trypanosoma cruzi* is well documented. What is less certain is whether all strains exhibit similar transmission patterns. This is especially of interest in North American versus South American strains of the parasite since in North America *T. cruzi* has evolved with placental mammals as the principal sylvatic reservoir hosts, while in South America marsupial species have dominated. Female BALB/c and outbred mice were infected with either the Brazil strain (BS), a Type I South American isolate, or a Type IIa isolate (SC) from North America. Breeding pairs were established and monitored for reproductive success. Pups were sacrificed at two weeks and tissues processed for diagnostic PCR using the TCZ primer set. BALB/c mice infected with the BS of *T. cruzi* failed to generate any off-spring. Those infected with the SC isolate produced off-spring in comparable numbers to those of uninfected control females. Of those pups born to LI infected female mice, 74% were PCR positive for *T. cruzi*. In the outbred mice 67% of the pups born to SC isolate infected females were positive (n=142), while from BS infected females only 33% of the

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pups (n=132) were infected. This data suggests that the Type IIa SC isolate is significantly more disposed to vertical transmission than the Type I BS. This is consistent with the co-evolution of this strain in placental mammals and possibly represents an increased reliance on this mechanism for transmission.

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THE ROLE OF ACRIFLAVIN IN THE INHIBITION OF TRYPANOSOMA MUSCULI GROWTH AND DEVELOPMENT BY INDUCING APOPTOSIS WITH SPECIFIC BINDING AFFINITY TO KDNA OF THE PARASITE *IN VIVO*

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Acriflavin is an intercalating agent and inhibitor of mitochondriogenesis. It also intercalates with the major and minor helices of DNA in bacteria, prokaryotes. Acriflavin treatment largely done *in vitro* resulted in destruction of the kinetoplast and resulted in respiratory defects. The binding affinity of acriflavin, its role in inducing apoptosis, its effect on the growth and development of *Trypanosoma musculi in vivo* were studied. The parasitemia levels at different time of infection, indicates that acriflavin has anti-trypanosomal and prophylactic activity *in vivo*. Gel shift assay showed the specific binding affinity of acriflavin to kinetoplast DNA (kDNA) both *in vitro* and *in vivo*. Furthermore the fluorescence microscope observation reveals the specific binding of acriflavin to the Kinetoplast DNA that was demonstrated by increased amounts of acriflavin in the kDNA relative to the control. The staining of fluorescent dye 4', 6-diamidino-2-phenylindole (DAPI) showed the effect of acriflavin in inducing fragmentation of kinetoplast DNA (kDNA). The fragmentation of the parasites' kDNA was further established using gel electrophoresis assay. The histological effect of the acriflavin on kDNA of the parasite was studied using transmission electron microscopy. Western blot analysis showed the release of cythochrome C from the kDNA to the cytoplasm and the subsequent activation (cleavages) of caspase 3 and 9 proteins. The membrane potential difference of the kDNA between the control and acriflavin treated parasites indicates its interference on the respiratory chain of the parasite which affects the ATP production activity. This study suggests that acriflavin treatment causes swelling of the kinetoplast and the condensation of the kDNA, which leads to death of the trypanosome.

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MEMORY CHARACTERISTICS OF PARASITE-SPECIFIC CD8+ T CELLS DURING CHRONIC AND DRUG-CURED EXPERIMENTAL *TRYPANOSOMA CRUZI* INFECTION

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Introduction of *Trypanosoma cruzi*, the causative agent of Chagas disease, into its mammalian host initiates a complex immune response capable of controlling parasitemia in the acute phase but unable to completely clear the infection, resulting in a life-long chronic infection. While studies have shown the CD8+ T cell response is essential for control of parasites during both the acute and chronic phases of infection, less is known about the generation and maintenance of the memory CD8+ T cell response during *T. cruzi* infection. In this study we used the C57BL/6 mouse model of *T. cruzi* infection to determine the parasite-specific memory CD8+ T cell response to IL-7 and IL-15, two cytokines critical for the survival of memory CD8+ T cells after pathogen clearance in viral and bacterial infection models. To identify parasite-specific CD8+ T cells, we used MHC class I tetramers containing the immunodominant *trans*-sialidase peptide, TSKB20. The majority of TSKB20-specific CD8+ T cells from mice with chronic *T. cruzi* infections did not express the high affinity IL-7Rα chain (CD127), the receptor required for responsiveness to IL-7. TSKB20-specific CD8+ T cells also failed to proliferate in the presence of IL-7 and IL-15 *in vitro*, consistent with the observed response of T cells in models of chronic viral infection. We also used an experimental model of *T. cruzi* infection in which the drug benznidazole was used to cure C57BL/6 mice infected with *T. cruzi*. Parasitological cure resulted in a change in phenotype of the majority of TSKB20-specific CD8+ T cells to high expression of CD127. These results suggest that *T. cruzi*-specific CD8+ T cells in infected mice fail to develop bona fide central memory characteristics as a result of persistent antigen exposure during *T. cruzi* infection. However in the absence of parasites, a CD127-expressing central memory CD8+ T cell population emerges.

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NEUTROPHIL INFILTRATION IS ASSOCIATED WITH INITIAL PARASITE CONTROL BUT SUBSEQUENTLY CONTRIBUTES TO TISSUE DAMAGE IN HAMSTERS INFECTED WITH LEISHMANIA (VIANNIA) PANAMENSIS

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We have established previously that upon infection with *Leishmania (V.) panamensis*, neutrophils from male hamsters are associated with higher parasite burdens and more severe lesions than resistant female hamsters. In this work we evaluated the role of neutrophils in male hamsters inoculated intradermally in the snout with 10e6 stationary phase promastigotes. Following *L. panamensis* inoculation there were distinct peaks in the number of intralesional neutrophils at 6 h and 21-25 days p.i (subacute phase). Depletion of neutrophils before the first peak (6 h) using either polyclonal antibodies or cyclophosphamide resulted in a 5.2 and 6.4 fold increase, respectively, in the percentage of infected macrophages infiltrating the inoculation site at 6 hours p.i. compared with controls ($p<0.001$). On the contrary, systemic administration of fMLP (formyl-Met-Leu-Phe), which increased the number of neutrophils during the first peak, resulted in a 2.4 and 8.4-fold decrease in parasitized macrophages at the inoculation site at 6 h and 19 days p.i., respectively ($p<0.001$). Depletion of neutrophils at the time of the second peak was associated with diminution of both the lesions ($p<0.001$) and proportion of amastigote-laden macrophages (6.5 to 7.9-fold reduction; $P<0.001$) at 22 and 45 days p.i. In contrast, at the latter time point (45 days p.i.), hamsters with increased numbers of neutrophils showed larger lesions ($p<0.001$) and higher amastigote densities (1.8 fold increase, $p<0,001$) than controls. These findings indicate that in this model of chronic cutaneous leishmaniasis, neutrophils participate in the initial control of *Leishmania*, but subsequently promote macrophage infection and lesion development.

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ADJUVANT EFFECT OF GARLIC EXTRACT WITH A DNA VACCINE AGAINST LEISHMANIA

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Leishmaniasis is a group of diseases caused by protozoa of diverse species of the genus *Leishmania*. A DNA vaccine encoding NH36 partially protected BALB/c mice against *L. mexicana* and *L. chagasi* infection. Garlic extract was also found to be a potent immunomodulator for the therapy of *L. major* and *L. mexicana* infection. We thus tested here the adjuvant effect of garlic extract with the NH36 DNA vaccine against *L. mexicana* and *L. chagasi* infection. Garlic bulbs were dried, extrated with water and the solution was filtered. The DNA vaccine VR1012-NH36 was produced from a culture of E. coli. BALB/c mice were vaccinated with VR1012-NH36

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DNA with or without dry garlic as adjuvant, and infected with L. mexicana or L. chagasi. The progress of the infection was evaluated by the size of the lesions measured by vernier or the parasitic load by Units of leishman donovan (LDU). The type of immune response was determined by phenotyping T lymphocytes by flow cytometry, measuring the production of IFNγ, delayed type hypersensibility (DTH), and antibody levels. Mice vaccinated with VR1012-NH36 plus garlic extract and infected by L. mexicana developed lesions larger than those that received the vaccine alone. The two doses of garlic extract tested together with VR1012-NH36 against *L. chagasi* infection did not improve protection, and while garlic alone potentiated the immune response, it did not affect parasite load. Thus, this study confirms the efficacy of the DNA vaccine alone, against both *Leishmania* species, and indicates that garlic extract has no adjuvant effect with the NH36 DNA vaccine.

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IDENTIFICATION OF NEW LEISHMANIA VACCINE CANDIDATES BY BIOINFORMATIC ANALYSIS OF LEISHMANIA MAJOR GENOME AND *IN VIVO* VALIDATION

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Leishmaniasis is a worldwide disease with an estimated 12 millon infected people and the population at risk is 350 millon. The recent completion of sequencing of *Leishmania major* genome together with bioinformatic tools open opportunities for the rational development of vaccines and identification of antigens. The objective of this work was thus to identify new vaccine candidates by bioinformatic analysis of the *L. major* genome and validate *in vivo* their immunogenicity. 8,272 translated sequences from the annotated *L. major* Friedlin genome were analyzed with RANKPEP epitope prediction algorithm to predict MHC class I mouse epitopes (H2-Kd and H2-Dd alleles). A total of 627 genes containing epitopes with MHC binding scores >85% were reanalyzed with additional epitope prediction programs to established consensus predictions, using 5 distinct algorithms for H2-Dd and 8 for H2-Kd. 79 genes with top consensus predictions (4/5 or 8/8 for H2-Dd and H2-Kd, respectively) were further analyzed to identify sequences conserved in other trypanosomatidae and eliminate sequences conserved in human and mouse genomes. Most of these genes encode for hypothetical proteins (64/79), and only 15/79 have a putative function. Interestingly, only 6/79 seem to be membrane-associated or surface proteins, and the remaining are intracellular. 20 of the top scoring genes were selected for validation *in vivo*. Transcriptionally active PCR products are being prepared for direct immunization of BALB/c mice and evaluation of their immunogenicity by flow cytometry and cytokine assays. We expect to be able to identify new potential vaccine antigens with this strategy.

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RELATIONSHIP OF POLYMORPHISMS IN *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN AND *P. VIVAX* PARASITEMIA AND SUSCEPTIBILITY TO RE-INFECTIONS IN A PROSPECTIVE COHORT STUDY OF PAPUA NEW GUINEAN CHILDREN

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The interaction between the *Plasmodium vivax* (Pv) merozoite Duffy binding protein region II (PvDBPII) and the human erythrocyte Duffy antigen (DA) is required for blood stage infections. However, the PvDBPII is highly polymorphic. We hypothesized that this polymorphism arose to enhance binding to the DA and therefore increase parasitemia levels or to avoid host immunity. To test this hypothesis, we followed 206 Papua New Guinean Children (median age= 9, 4-14 years) bi-weekly for six months following eradication of blood stage infections. 94% of the children were re-infected based on PCR diagnosis with Pv representing 736 positive samples during the 6 months children were followed. Age and pre-existing Pv infection were significantly associated with time to first re-infection and incident density parasitemia. Twenty-seven different PvDBPII haplotypes consisting of 14 polymorphic loci were observed during the study, although three haplotypes represented 57% of all Pv infections. The relative frequencies of PvDBPII haplotypes were similar at all time points during the study. We found no correlation between the PvDBPII haplotypes with Pv parasitemia. Based on the three most prevalent PvDBPII haplotypes, we found no association between the presence of the haplotype at the beginning of the study and the presence of that haplotype upon re-infection. Thus, our initial analysis failed to show that polymorphisms in *PvDBPII* affects parasite virulence or are related to development of strain-specific immunity in older children where significant immunity to Pv has been established.

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P. FALCIPARUM FCR3ΔVAR2CSA MUTANTS- A NOVEL TOOL TO EVALUATE PARASITE LIGANDS INVOLVED IN PLACENTAL MALARIA

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In high transmission regions, protective clinical immunity to *Plasmodium falciparum* develops during early years of life, limiting serious complications of malaria to young children. Pregnant women are an exception and are especially susceptible to severe *P. falciparum* infections resulting mainly from the massive adhesion of *Plasmodium falciparum* infected erythrocytes (IE) to placental syncytiotrophoblasts. Chondroitin sulfate A (CSA) present in the placental intervillous blood spaces has been described as the common receptor involved in the massive sequestration of IE to the placenta. However, it is controversial if other receptors are involved in placental sequestration. We previously reported that disruption of a particular member of the var gene family (var2csa) results in the inability of the FCR3Δvar2csa parasites to recover binding to CSA. In this study we used our FCR3Δvar2csa mutant to investigate if the parasite genome encodes for adhesion molecules that bind to non-CSA placental receptors. Experimental evidence suggested that placental parasites could adhere to hyaluronic acid (HA). Multiple rounds of selection of the FCR3Δvar2csa IE on bovine HA did not result in the selection of HA binding IE, whereas FCR3 wild-type parasites selected on bovine HA resulted in the selection of IE with the CSA- and HA-binding phenotypes. In order to verify the HA binding specificity, different sources of HA were used and revealed that the observed binding results from CSA contamination. Multiple panning on the placental derived BeWo cell line resulted in the emergence of parasites able to cytoadhere in a CSA-independent manner. We are currently investigating the parasite ligand and its role in pregnancy-associated malaria.

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CHONDROITIN 4-SULFATE MEDIATED ADHERENCE OF *PLASMODIUM FALCIPARUM* IN PREGNANCY-ASSOCIATED MALARIA: DESIGN OF NOVEL PHOTOACTIVABLE REAGENTS FOR THE IDENTIFICATION OF PARASITE ADHESIVE PROTEINS

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A general feature of *Plasmodium falciparum* is the adherence of infected red blood cells (IRBCs) in the vascular capillaries of various organs, leading to cerebral and other organ-related malaria illness. In endemic areas people by adulthood regardless of gender produce inhibitory antibodies to the adhesive proteins and thus avoid IRBC adherence and malaria pathogenesis. However, in the case of women, during pregnancy, the IRBCs with adherent specificity to chondroitin 4-sulfate (C4S) selectively adhere in the placenta causing pregnancy-associated malaria. Our previous studies have shown that a very low sulfated chondroitin sulfate proteoglycan (CSPG) is the receptor for IRBC adherence in placenta. Here, we performed a comprehensive study to determine the structural basis for IRBC adhesion to C4S. Replacement of the *N*-acetyl groups with bulky *N*-acyl or *N*-benzoyl substituents had no effect on the inhibitory activity of C4S, whereas reduction of the carboxyl groups abrogated the activity. Dermatan sulfates showed ~50% inhibitory activity when compared to C4S with similar sulfate contents. These results indicate that the C4S carboxy groups and their equatorial orientation are critical for IRBC binding, whereas the *N*-acetyl groups are not required. Conjugation of bulky substituents to the reducing end *N*-acetylgalactosamine residues of C4S dodecasaccharide had no effect on the inhibitory activity of the oligosaccharide. Based on these results, we prepared photoaffinity reagents for the identification of the IRBCs adherence protein(s). Crosslinking of the IRBCs with a radio-iodinated photoactivable C4S dodecasaccharide, labeled a low molecular weight parasite protein on the IRBC surface, suggesting a novel protein is involved in C4S binding. Conjugation of biotin to the C4S dodecasaccharide photoaffinity probe afforded a strategy for the isolation of the labeled protein by avidin-affinity precipitation, facilitating efforts to identify the C4S-adherent IRBC protein(s).

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MK2 AND P38 MAPKS DIFFERENTIALLY REGULATE THE IL-12 PRODUCTION IN MACROPHAGES STIMULATED WITH GLYCOSYLPHOSPHATIDYLINOSITOLS (GPIS) OF *PLASMODIUM FALCIPARUM* THROUGH DIFFERENT MECHANISMS

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Glycosylphosphatidylinositols (GPIs) of *Plasmodium falciparum* are thought to contribute to malaria pathogenesis by inducing the production of proinflammatory mediators, such as TNF-α, IL-6, IL-12, and nitric oxide in the host. The GPI-induced production of proinflammatory responses in macrophages is mediated mainly through the recognition of TLR2, engaging the downstream activation of ERK, p38, JNK MAPKs and NF-κB pathways. The activation of p38 MAPK pathway is critical for the GPI-induced production of IL-6 and IL-12, whereas this pathway is only marginally involved in the expression of TNF-α and nitric oxide. Here, we found that MAPK activation kinase 2 (MK2 or SAPK2) plays an important role in the cytokine regulation. Although, MK2 is widely thought to be solely under the control of p38 MAPK, p38 and MK2 play differential roles in the GPI-induced production of various cytokines. Blocking of p38 MAPK activation leads to a marked inhibition of IL-12 production in macrophages, whereas lack of MK2 gene expression results in 2-fold higher level of IL-12 production. In contrast, inhibition of p38 caused only <20% decrease in TNF-α level by macrophages, whereas lack of MK2 caused ~50% reduction in the level of this cytokine. Both MK2 and p38 are crucial for the stability of mRNA of various cytokines, but they differentially regulate the cytokine expression. The p38 MAPK positively regulates the IκBζ gene induction and its nuclear translocation, and NF-κB binding, the events that are involved in IL-12 expression, whereas, MK2 has no effect on these events. Further, lack of MK2 expression leads to IL-12 upregulation by enhancing κB binding to the IL-12p40 promoter, and by reducing the level of nuclear inhibitory factor c-Maf and decreasing the binding of GAP-12 to IL-12p40 promoter.

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SICKLE-CELL TRAIT (HBAS) IS ASSOCIATED WITH DECREASED DEPOSITION OF MALARIAL PIGMENT (HEMOZOIN) IN MONOCYTES OF CHILDREN WITH ACUTE *PLASMODIUM FALCIPARUM* MALARIA IN WESTERN KENYA

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Sickle hemoglobin (HbS) results from a single point mutation (glutamic acid to valine) in the β subunit of hemoglobin. A high frequency of the HbS variant is likely maintained in sub-Saharan Africa because heterozygous carriers (HbAS) have increased resistance to *Plasmodium falciparum* malaria. We have previously shown that HbAS protects against childhood severe malarial anemia (SMA; Hb<6.0 g/dL), but not high-density parasitemia (HDP; 10,000 parasites/μL or greater) in a *P. falciparum* holoendemic area of western Kenya. Our investigations in this region further revealed that elevated numbers of pigment (hemozoin, Hz)-containing monocytes (PCM) are significantly associated with enhanced development of SMA. To extend these findings, we determined if HbAS was associated with protection against SMA by decreasing Hz acquisition by monocytes and neutrophils in children with acute malaria (n=618, aged <3 yrs). Multivariate logistic regression controlling for age, gender, and HIV-1 status, revealed that children with HbAS had reduced numbers of PCM compared to children with normal Hb alleles (HbAA) [0.51 (0.32-0.83); p<0.01]. Stratification of children into low (10% or less) and high (greater than 10%) numbers of PCM further demonstrated that HbAS protected against both low [0.56 (0.34-0.94); p<0.05] and high [0.09 (0.01-0.70); p<0.05] levels of PCM. However, no significant relationships were found between Hb genotypes and the number of pigment-containing neutrophils. Taken together, results here show that HbAS-mediated protection against SMA may be due, at least in part, to decreased monocyte-acquisition of Hz. Since accumulation of Hz occurs during the late stages of the *Plasmodium* life cycle (i.e., late trophozoites and schizonts), we hypothesize that carriage of the HbS variant may reduce Hz deposition by promoting immune recognition and clearance of parasites during early stages of the erythrocytic cycle (i.e., rings and early trophozoites) where Hz synthesis is minimal.