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T cell (CTL) killing of autologous B lymphocyte cell lines infected with a recombinant vaccinia virus expressing WNV E. T cell lines were generated by limiting dilution. We identified three novel human CD8 T cell epitopes contained within WNV E protein, restricted by HLA A*0201, HLA A68 and HLA B18, respectively. In a cohort of 19 HLA A2 individuals who received this vaccine, all developed WNV E A2 epitope-specific T cells detected by HLA tetramer staining. The frequency of CD3+CD8+ epitope-specific cells ranged from 0.1-2.6%. Peak tetramer frequencies were measured on day 14 in 5 (26%) and on day 28 in 14 (74%) of these individuals. We detected WNV E-specific CTL in 13 of 19 (68%). Peak CTL responses occurred on day 14 or 28 post-vaccination. Tetramer positive cells were detected at day 90, 180 and/or 360 in 15/17 (88%) of HLA A2 positive individuals for whom late samples were available. These data demonstrate that the YF backbone of this novel chimeric vaccine did not interfere with the development of robust CD8 T cell responses to the foreign gene (WNV E) in humans immunized with a novel chimeric virus vaccine. A West Nile virus vaccine that induces durable WNV-specific CD8 T cell responses as well as neutralizing antibodies is likely to be effective in protecting the host from disease.

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A RECOMBINANT WEST NILE SUBUNIT VACCINE PROVIDES EFFECTIVE PROTECTION AGAINST FATAL WEST NILE ENCEPHALITIS IN AGED AND WEANLING HAMSTERS

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Most human cases of West Nile disease are asymptomatic or result in a flu-like illness (West Nile fever), however, about 30-40% of cases reported to the Centers for Disease Control and Prevention in the last few years had severe neurological symptoms. Approximately 5-15% of the latter cases are fatal, and a high percentage of the non-fatal cases result in permanent neurological disabilities. Moreover, the fatality case rate is about 30% in victims over the age of 70. Currently, there is no approved commercially available vaccine for prevention of West Nile virus infection in humans, nor any specific therapy for disease. Hawaii Biotech has used a recombinant subunit approach for development of several flavivirus vaccines, including West Nile virus. The vaccines include envelope (WN-E) with or without non-structural (WN-NS) proteins produced in an insect cell expression system and purified by immunoaffinity chromatography. Low dose (1 mcg WN-E) vaccine formulations were found to completely protect (100% survival) golden hamsters against lethal West Nile encephalitis after challenge with live virus. Moreover, vaccinated animals remained free of clinical disease after challenge and had no detectable viremia. High titers of viral neutralizing and other antibodies were also elicited by the vaccine in hamsters (pre-challenge). Two major questions remained about the efficacy of a subunit vaccine approach for West Nile disease. One question was whether the vaccine would elicit a durable immune response. The other question was whether the vaccine would elicit a protective immune response in weanling and aged animal models. Protection (100% survival) against lethal viral challenge was demonstrated for at least 12 months beyond booster vaccination with a lack of viremia as well. These results show the vaccine to elicit a durable immune response. Viral neutralizing antibodies remained stable for this entire time. To answer the question of vaccine efficacy in young and old animals, both aged hamsters (12 months old at primary vaccination) and weanling hamsters (3 weeks old at primary vaccination) were shown to be completely protected against lethal challenge by low dose WN-E vaccination as well. This was shown with either of two different saponin-based adjuvants in the case of the aged animals. Viremia post challenge was reduced by 4-5 logs compared to adjuvant control aged animals, and high titers of antibodies were also produced (pre-challenge).

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INVESTIGATION INTO THE COMPARATIVE EFFICACY OF THREE WEST NILE VIRUS (WNV) VACCINES IN EXPERIMENTALLY INDUCED WNV CLINICAL DISEASE IN HORSES

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This is the first report of a comparative vaccine efficacy trial in horses where West Nile Virus (WNV) induced encephalomyelitis was demonstrated in all of the control animals. These studies demonstrate differences in the abilities of two commercial vaccines and a live chimera vaccine to protect against West Nile Virus induced disease at 28 days post-vaccination in horses. Since its emergence into the United States in 1999, West Nile Virus has killed a total number of 785 humans, 6,000 horses, and an estimated hundreds of thousands of birds. This mosquito-borne encephalitic flavivirus has caused clinical disease in 19,444 humans and 22,908 horses.[2] Using a reproducible model of WNV induced encephalomyelitis[3], the efficacy of a new, live, chimera vaccine and two commercially available vaccines for prevention of clinical signs of West Nile virus (WNV) induced disease was tested in horses. Animals. Twenty-four healthy, WNV-seronegative horses of varying ages and gender, were randomized into 3 blinded vaccination/challenge trials. Horses were maintained according to University of Florida Animal Care Services and IACUC guidelines. Efficacy Trials. Horses were placed into three randomized, blinded trial groups consisting of 8 horses, with two horses in each group receiving: 1) a killed WNV vaccine, 2) a modified-live vaccine containing WNV prM and E proteins expressed by a canarypox vector, 3) a live chimera vaccine containing WNV prM and E proteins expressed by a YF17D vector, or 4) a diluent. For the commercial inactivated and modified-live vaccines, horses received an initial primary injection followed in 28 days with a second injection according to manufacturer's label. For the chimera vaccine, each horse received a primary injection dose of diluent followed in 28 days by a single vaccine dose. All horses were challenged intrathecally with virulent WNV 28 days after vaccination. Monitoring of Animals. Complete physical and neurological evaluations were performed for 21 days post challenge (PC). Horses were observed for clinical signs and neurological signs of mentation, fasciculations, paresis, ataxia, and cranial nerve abnormalities.[4] Horses were euthanized for humane reasons due to overall severe health condition of the animal as a result of WNV infection and/or the inability to locomote without assistance. Twenty-one days PC, all remaining horses were euthanized and a full gross and histopathological evaluation was performed. Cross sections of the brain and spinal cord were examined and quantified for the presence of gliosis and perivascular cuffing. Serum and plasma samples were collected before and after challenge for virological evaluation. Statistical Analysis. Neurologic signs were analyzed as 0 = none and 1 = present. Data from all 3 trials were combined for X^2 and Fischer Exact analysis and clinical signs > 0 and present for > 1 day. Level of significance was set at $P < 0.05$. Statistical analysis was performed by use of computer spreadsheet (Excel, Microsoft, Redmond, WA) statistical analysis package (SysStat, Point Richmond, CA). Three of 6 control horses developed increased rectal temperature (102.5°C) and 6/6 developed PC viremia, grave neurological disease, and were euthanized for humane reasons before the end of the trial. Gross and histopathological changes consistent with WNV polioencephalomyelitis were observed in all control horses. None of the vaccinates, irrespective of the vaccine administered, developed PC viremia, and all survived to the end of the trial period, at which time a full gross and histopathological evaluation was performed. For the chimera group, none of the vaccinates developed increased rectal temperatures and no neurological signs were observed. One of the horses given the modified-live vaccine was determined to have pre-existing neutralizing antibody to WNV and was removed from the study. For the modified-live vaccinates, 1/5 horses developed increased rectal temperatures and 1/5 had consistent mild neurological signs in several categories. Another had mild malaise and anorexia after challenge. For

horses given the inactivated vaccine, 1/6 developed increased rectal temperature and 4/6 develop mild to moderate neurologic signs that occurred relatively late in the challenge period. Chimera vaccinates demonstrated significantly less clinical signs than horses vaccinated with the inactivated vaccine ($p \leq 0.035$) or control animals ($p \leq 0.01$). Histopathological analysis demonstrated moderate to severe changes in the controls consistent with WNV encephalitis and mild changes in some vaccinated horses. Using a severe challenge model of WNV that induced encephalomyelitis, the efficacy of a new, live, chimera vaccine and two commercially available vaccines for prevention of clinical signs of West Nile virus (WNV) induced disease was tested in horses. Challenge of horses by this model caused grave and reproducible neurological signs in all six horses that were not vaccinated. In contrast vaccination with one dose of the chimera vaccine or two doses of the commercial inactivated or modified-live vaccines resulted in 100% survivorship. Horses in both the inactivated and modified-live vaccine groups had a higher occurrence of clinical signs PC when compared to the chimera group. The efficacy of these vaccines against WNV induced disease in horses challenged >28 days post-vaccination is not known. Additional studies with larger sample sizes and at longer durations after initial vaccination are warranted.

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DISCOVERING NOVEL BLOOD STAGE MALARIA VACCINE CANDIDATES: SCREENING WITH IMMUNE SERA FROM FALCIPARUM MALARIA PATIENTS AND ASYMPTOMATIC PARASITE CARRIERS

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Completion of the reference genome sequence of *Plasmodium falciparum* has opened the way to discovery of novel malaria vaccine candidates. There are several lines of evidence from studies of naturally acquired immunity that immune response to the defined blood stage antigens is associated with protection against either malaria infection or clinical malaria, which makes the development of asexual blood stage vaccines feasible especially for residents in endemic areas. Here we took steps towards getting schizont-merozoite stage antigen proteins: creating customized and manageable set of genes for cloning according to the reports on transcriptome and proteome, and synthesizing recombinant protein from ~150 genes by a wheat germ cell-free system. These recombinant proteins were then screened for reactivity to immune sera from malaria exposed Thai individuals. The sera samples used are divided into two groups; from those hospitalized with the past history of falciparum malaria, or from the residents without clinical symptoms (asymptomatic parasite carriers) in continuously-monitored cohort village. The data processing after analysis of individual sera samples marked the difference between these two immune groups in the reaction pattern against a panel of recombinant proteins including known blood stage vaccine candidates. This screening approach, combined with high-throughput protein expression, will help to prioritize antigen molecules for detailed study in terms of protective potential and eventually lead to novel asexual blood stage vaccine candidates involved in humoral immunity.

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DISCOVERING NOVEL MALARIA PRE-ERYTHROCYTIC ANTIGENS

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Naval Medical Research Center is developing a multi-stage malaria vaccine designed to prevent malaria infection and to reduce clinical manifestations of disease, especially severe disease or death, in those who become infected. The feasibility of developing such a vaccine is supported by the sterile protection induced in humans by the radiation-attenuated sporozoite vaccine, which prevents parasites from fully developing in the liver, therefore preventing the onset of the erythrocytic infection. Although unknown, the antigens inducing this protective immune response are thought to be expressed in both the sporozoite and in the infected hepatocyte stages of the parasite. We have previously identified and cloned a panel of *P. falciparum* genes putatively expressed in the sporozoite and/or hepatocyte stages of the parasite. The high throughput expression of these genes as recombinant proteins was successfully achieved by a modified Wheat Germ cell-free system; this protein expression platform consistently yielded over 80% expression efficiency, with the majority of expressed proteins in soluble form. Several of these proteins were then chosen by batch screening for reactivity to panels of immune sera from naturally immune individuals and from volunteers immunized with irradiated sporozoites. Following affinity purification of these recombinant proteins, we raised polyclonal mouse and rabbit sera for antigen characterization studies such as parasite stage expression, cellular localization and functional activity. The analysis of several of these sera by immuno fluorescence (IFA) and Western Blotting assays have identified proteins expressed in a wide range of parasite stages including eight expressed in sporozoite stages, three in liver stages, four in various erythrocytic stages and three in gametocyte stages. These and future studies will help to qualify the potential of these antigens to serve as vaccine candidates. The combination of the Wheat Germ cell-free protein expression and screening protocols greatly enhances our ability to identify pre-erythrocytic stage vaccine candidates by their reactivity to antibody and more importantly to T-cell immune responses.

(ACMCIP Abstract)

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CHIMERIC MSP-1 BASED VACCINE-INDUCED ANTIBODIES CROSS-REACT WITH SEVERAL PLASMODIUM SPECIES AND INDUCES PROTECTIVE IMMUNITY

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We have previously reported the design and use of linear polymeric synthetic peptide chimeras as viable delivery system for subunit malaria vaccines. Distinctive features of such synthetic chimeras are the inclusion of *Plasmodium* universal T cell epitope and their topology that facilitates extensive polymerization. To further assess the relevance of malaria universal T cell epitopes in vaccine design we constructed a synthetic gene that includes two universal T cell epitopes reported by us in *P. vivax* and located upstream from the P. yoelii MSP-1(19) fragment. The synthetic gene, codon optimized for expression in E. coli, also includes two different

tags used for protein purification and the assessment of antigenic integrity using monoclonal antibodies. Groups of BALB/c and C57BL/6 mice were used to test immunogenicity of the recombinant construct. A single immunization induces high antibody titers against both recombinant chimera and synthetic peptides. Specific antibody concentration for each isotype induced by immunization could be ranked as IgG1=IgG2a=IgG3>IgG2b for BALB/c mice and IgG2a>IgG1=IgG3>IgG2b for C57BL/6. These results suggest that different populations of T helper cells are induced after immunization with the recombinant construct. Protein and synthetic peptide-specific IFN-γ recall responses were identified in BALB/c mice after priming. This is in contrast with the predominant protein-specific IFN-γ recall response identified in C57BL/6. Interestingly, sera samples obtained from immune mice recognize recombinant proteins representing the two allelic forms of *P. falciparum* MSP-1(19) as well as native *P. falciparum* proteins on western blots. After experimental challenge mice were partially protected against infection and malarial anemia. Our results demonstrate the potential for developing complex recombinant chimeras containing parasite universal T cell epitopes as malaria vaccine candidates.

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TOLERABILITY AND IMMUNOGENICITY OF A *P. FALCIPARUM* MULTI-ANTIGEN MULTI-STAGE ADENOVIRUS VECTORED VACCINE, NAVAL MEDICAL RESEARCH CENTER-M3V-AD-PFCA, IN NZW RABBITS

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We have developed a candidate malaria vaccine, Naval Medical Research Center-M3V-Ad-PfCA, designed to induce cellular and humoral immune responses against both pre-erythrocytic and erythrocytic stages of the *P. falciparum* parasite life cycle. The vaccine is a cocktail of two Ad5 serotype adenovirus vectors (E1, E4, partial E3 deleted) expressing PfCSP and PfAMA-1. We tested Naval Medical Research Center-M3V-Ad-PfCA in a repeat-dose GLP study in NZW rabbits to evaluate its safety, toxicology and immunogenicity profile. The vaccine was administered at either the full anticipated human dose ($1x10^{11}$ pu in 1 mL) or a low dose ($2x10^{10}$ pu in 1 mL) intramuscularly on study days 1, 11, and 32. Animals were evaluated for mortality, clinical observations, body weight, food consumption, opthalmology, body temperature, dermal evaluation of injection sites, clinical pathology, gross pathology, organ weights, histopathology, and malaria-antigen specific immune responses. Immunogenicity was evaluated pre-study, 2 days post each vaccine administration, and at necropsy. Necropsy evaluations were 2 days and 14 days post-final vaccine administration. There was no apparent systemic toxicity. Occassional transient increases in body temperatures at 3 and 24 hours post vaccine administration returned to normal prior to the subsequent dose. Naval Medical Research Center-M3V-Ad-PfCA-dosed animals developed a minimal to mild erythema/edema and a minimal to mild inflammatory response at the injection site. Minor changes in globulin, cholesterol, triglycerides, and albumin to globulin ratio were considered part of the inflammatory response. These responses resolved and did not suggest clinically relevant adverse effects. There were no adverse effects on mortality, clinical observations, dermal injection site evaluation, body weights, food consumption, opthalmologic findings, gross pathology or histopathology. PfCSP- and PfAMA-1- specific antibodies were detected in the rabbit sera, demonstrating that Naval Medical Research Center-M3V-Ad-PfCA was immunogenic. Overall, these data support the clinical evaluation of Naval Medical Research Center-M3V-Ad-PfCA.

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PRE-CLINICAL STUDIES TOWARDS RAD35-BASED MALARIA VACCINES

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Each year more than 1 million people succumb to malaria and therefore a safe and effective vaccine is a global health priority. Evidence thus far suggests that an effective vaccine against malaria should elicit potent antibody and T-cell responses, which adenoviral vectors in general are able to induce. However, clinical utility of commonly used rAd5-based vaccines may be hampered by wide spread pre-existing anti-Ad5 immunity in human population. Here we show that human adenovirus type 35 (rAd35) has low sero-prevalence worldwide including countries where malaria is endemic. We demonstrate that immunization with rAd35.CS vaccine resulted in strong T-cell responses against *P. yoelii* CS and protection upon high dose *Plasmodium yoelii* challenge in either naïve mice or mice carrying high level anti-Ad5 neutralising activity. Furthermore, we show that an rAd35.CS vaccine induced strong T-cell responses in macaques against the *P. falciparum* derived CS insert. Although clinical trials will eventually establish the utility of this vaccine, we further investigated the potency of a rAd35.CS vaccine in a near-physiological human skin model. Results of these experiments and data concerning further exploration of improved adenovirus based vaccines will be discussed.

(ACMCIP Abstract)

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IMMUNOGENICITY AND PROTECTIVE EFFICACY AGAINST PLASMODIUM VIVAX IN AOTUS MONKEYS FOLLOWING HETEROLOGOUS PRIME-BOOST IMMUNIZATION WITH PLASMIDS AND ADENOVIRUS VECTORS ENCODING PVAMA1 AND PVMSP1-42

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We have evaluated the immunogenicity and protective efficacy of heterologous DNA prime/adenovirus boost vaccine regimens in the *Aotus lemurinus lemurinus* malaria blood stage challenge model. Coding sequences for blood stage antigens PvMSP1-42 and PvAMA1 (Sall strain) were inserted into separate plasmid and adenoviral (Ad5) vectors. DNA alone, Ad5 alone, and DNA prime-Ad5 boost regimens were tested for the antigens in combination, and the DNA prime/Ad5 boost regimen for each antigen individually. For prime/boost regimens, three intradermal injections of DNA were administered at four week intervals at doses of 500 ug per plasmid (single-antigen groups) or 250 ug per plasmid (combined-antigen groups), followed fifteen weeks later by a single, intradermal Ad5 boost of 1e10 vp (viral particles) per construct (single antigen groups) or 5e9 vp per construct (combined antigen groups). The same regimens were used when the prime or the boost was administered alone, except that empty vectors were administered in place of the omitted immunization. Five weeks after the last immunization, all animals plus five unimmunized infectivity controls were challenged by intravenous administration of 10,000 parasitized erythrocytes in 1 mL RPMI 1640. Development of parasitemia was followed daily from days 1-35 post-challenge. The DNA prime/Ad5 boost regimens of PvAMA1 alone, PvMSP1-42 alone, or their combination, and, to a lesser extent, DNA prime by itself utilizing both antigens, protected the majority of animals, as measured by the ability to self-cure,

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while Ad5 boost alone utilizing both antigens was not protective. The course of parasitemia, hematology, and humoral immune responses to the vaccine regimens as measured by ELISA using recombinant proteins and IFA against whole parasites, will be presented.

(ACMCIP Abstract)

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EFFECTIVE BOOSTING VECTORS FOR MALARIA IMMUNIZATION EVADE THE CD8 T CELL RESPONSE GENERATED BY PRIMING

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Prime-boost regimes are required for the induction of robust CD8 T cell responses by vaccination. Much descriptive work has been done to describe possible combinations of priming and boosting vectors. However little is known about the requirements for a robust recall response and, by extension, why some vectors boost and others fail. We tried boosting a *Plasmodium yoelii* sporozoite specific CD8 T cell response with recombinant flu, vaccinia and adenovirus vectors expressing the malaria circumsporozoite (CS) protein epitope SYVPSAEQI. Only recombinant vaccinia was able to effectively boost the immune response. The ability of vaccinia to boost was found to be independent of CD4 T cells but absolutely dependent on dendritic cells. We compared the abilities of the different vectors to prime naïve transgenic CD8 T cells specific for the CS epitope in the presence of pre-existing sporozoite immunity. Only vaccinia was able to overcome the preexisting immunity and prime the transgenic cells. In contrast flu and adenovirus vectors were unable to prime naïve cells in the presence of antigen specific memory CD8 T cells. This suggests that vaccinia is an effective boosting vector because it is able to carry antigen for presentation in the face of the memory CD8 response generated by priming.

(ACMCIP Abstract)

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NATURE BEATS NURTURE: A CASE STUDY OF THE QUALITY OF MALE *ANOPHELES GAMBIAE* S.L MOSQUITOES REARED IN ARTIFICIAL AND NATURAL ENVIRONMENTS.

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Laboratory experiments form the majority of our knowledge of the behaviour, physiology and ecology of many organisms; and in particular insects. However, the accuracy with which lab-derived estimates of insect life history and behaviour can predict their fitness and population dynamics in the wild is rarely validated. Such comparison is especially essential in cases where lab-derived information is used to formulate environmentally-conducive strategies for insect control. Here we conducted a comparative study of the reproductive potential and life-history of male *Anopheles gambiae* s.l mosquitoes under optimal lab conditions and at ambient conditions in an area of intense malaria transmission in East Africa. We measured three indirect indicators of male mosquito fitness: energetic reserves, body size, and survival, in a bid to understand if the demographics and energetic limitations of wild males can be correctly predicted from their lab counterparts. Crucially, we found that the body size and lipid stores of wild males were substantially greater than those from ideal lab conditions. These results infer that simplified lab environments underestimate the nutritional benefits that wild mosquitoes can obtain from foraging freely. We caution that the energetic limitations of insects as identified in the lab may underestimate their resilience in the wild, and discuss the implications of this phenomenon with respect to malaria control programmes based on releasing genetically modified male mosquitoes.

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MALE *ANOPHELES GAMBIAE* MATING SUCCESS IN A SWARM: 'MAY THE BEST MAN LOSE'

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The use of genetically manipulated (GM) and /or sterile male mosquitoes for malaria control is becoming a possibility. The success of such programmes requires better understanding of male mating biology and ultimate mating competitiveness. It has been observed that factors such as body size and energetic reserves are reliable indices of mating success in female *Anopheles gambiae* mosquitoes and other flying insects. Here we investigated the influence of these traits on male mating success by manipulating larval nutrition. We hypothesized that male quality as indexed by nutrition-dependent body size and energetic reserves would be a reliable indicator of mating competitiveness in a swarm. Male *An. gambiae* larvae were reared at high, intermediate and low food conditions to generate adult males that differed in three typical indices of mating fitness: body size, energetic reserves, and survival. Resulting adult males were competed against one another for access to females. When competed against one another in swarms, males from the intermediate food treatment were 10 times more successful in obtaining females than those from the highest food treatment (Odds ratio [95% CI]=10.33 [2.1-19.52]), and 3 times more competitive than males from the low treatment (Odds ratio [95% CI] = 3. 93 [0.93 - 4.71]). Body size, reserve provisioning and survival could not explain the success of the intermediate group. Instead we observed that males from the intermediate treatment were closest in body size to females, and hypothesize that phenotypic similarity of males to females is a more important predictor of their mating competitiveness than overall quality. This observation of assortative mating between *An. gambiae* of similar phenotypes has important implication for control programmes aiming to reduce malaria transmission by mass releasing sterile and /or genetically or phenotypically modified males. In order for released males to be competitive, they must be reared in backgrounds as similar as possible to wild-type females.

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DIFFERENTIAL SEGREGATION OF MATERNAL LIPIDS AS A STRATEGY FOR NEONATE LARVAE SURVIVAL IN THE MOSQUITO

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In animals, lipids are a source of energy, cell membrane components, signaling pathway modulators, and emulsifying agents. Because lipids serve functionally different purposes, segregation of lipids in appropriate tissues is essential for the birth of healthy neonates. We found a novel role of maternal lipids in newly hatched mosquito larvae. To deliver traceable lipids analogs into oocytes, we used purified lipophorin from adult An. gambiae. We found that the lipophorin delivers the lipids to the developing oocyte in an energy-dependent, receptor-mediated process. When oocytes from these mosquitoes were examined, fatty acids

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and phospholipids were found to be co-localized within the same yolk bodies. To investigate how imported lipids are distributed in embryonic mosquito larvae, we collected eggs from mosquitoes that were injected with a mixture of phospholipids and fatty acids. When the eggs hatched, both the phospholipid and fatty acid analogs were found in the neonatal larvae. Distribution of the lipid types, however, was notable in these newly hatched larvae. A significant portion of the fatty acid segregated in spherical vesicles in the thorax, and along the side of the body cavity. This indicated that in addition to providing resources for biosynthesis of cellular components, a portion of fatty acids are stored along the body cavity and thorax for unknown purposes. In contrast, imported phospholipid was segregated differently inside the neonate intestine and in the gastric caeca. This unique segregation of the fatty acids and the phospholipids was reproducible in all repeated experiments. These observations indicate that newly hatched larvae need the maternal lipids to use even after emergence. We will discuss the implications this unusual segregation of maternal lipids has on the survival of newly hatched larvae. We will also discuss the implications these findings have to developing novel strategies of mosquito control, to reduce the burden of mosquito-borne diseases.

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DEFORESTATION AND ITS EFFECTS ON THE SPOROGONIC DEVELOPMENT OF *PLASMODIUM FALCIPARUM* IN *ANOPHELES GAMBIAE* IN THE HIGHLANDS OF WESTERN KENYA

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This study investigated the effects of microclimatic changes caused by deforestation on the sporogonic development of *Plasmodium falciparum* in *Anopheles gambiae* in an epidemic prone area of the western Kenya highlands. *A. gambiae* mosquitoes were fed with *P. falciparum* infected blood through membrane feeders. Fed mosquitoes were divided into different cages and placed in houses in forested and deforested areas in the village of Iguhu, Kakamega, western Kenya (elevation, 1500 m above sea level). Indoor and outdoor temperature and relative humidity in forested and deforested areas were measured by a hobo data logger. Indoor maximum and mean temperatures in the deforested area were higher than those in the forested area by 3.6°C and 1.2°C respectively. Average time for oocyst to appear in mosquitoes was 0.9 days shorter in mosquitoes placed in the deforested area than those in the forested area (7.5 vs. 8.4 days; $P < 0.0001$). The average time for *P. falciparum* to develop to sporozoites was 1.1 days shorter in the deforested area than that in the forested area (12.8 vs 13.9 days; $P < 0.05$). Infection rates of mosquitoes placed in the deforested area were similar to the forested area (22.6 % vs.16.7%; $P>0.05$). This study showed that deforestation increases the indoor temperature and consequently decreases the sporogonic time of *P. falciparum* in *A. gambiae.* Reduced sporogonic development time by deforestation enhances vectorial capacity of *A. gambiae* and thus increases malaria transmission risk.

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COMPLEXITY OF VERTEBRATE BLOOD UTILIZATION IN *ANOPHELES GAMBIAE*: ROLES OF PROTEASES AND LIPASES

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Adaptation to utilize vertebrate blood has provided access to highly nutritious meals for mosquitoes. Indeed, anautogenous mosquitoes require vertebrate blood for reproduction and propagation. However, the adaptation also poses the challenge of evolving specialized digestive enzymes. Major enzymes of protein digestion are a number of trypsins and carboxydases. Trypsins are endopeptidases which cleave polypeptides internally at the C-terminus of only lysine and arginine residues. In contrast, carboxypeptidases are exopeptidases that non-specifically cleave from the C-terminus of any polypeptide. Vertebrate blood contains many diverse types of proteins with variable numbers of lysine and arginine residues, potentially resulting in differential kinetics of protein degradation. In addition, lipid-protein complexes in vertebrate blood, VLDL, LDL and HDL, with the kinetics of expression of diverse proteases and lipases, make vertebrate blood digestion a complex process. To gain a deeper insight into the initial stages of the blood digestion process, we have utilized density ultracentrifugation, specific mono and polyclonal antibodies, and protein digestion analysis *in vitro* and *in vivo*. We have also used the kinetics of lipid release from lipid-protein complexes after the density ultracentrifugation of human and mouse blood components. The study has provided a comprehensive look at the different dynamics of individual components of vertebrate blood in the midgut of the malaria vector with which the developing pathogens compete.

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SPECIATION BY ECOTYPIFICATION IN *ANOPHELES GAMBIAE*: A QUANTITATIVE TEST

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In his ecotypification hypothesis, M. Coluzzi describes how paracentric inversions may lead to Anopheline speciation through suppressed recombination. He suggests that suppressed recombination could prevent the homogenization of co adapted loci with maladapted alleles. His verbal model is supported by recent discoveries of genes under selection within inversions and in other areas of reduced recombination, but it has not previously been subjected to a quantitative test. The large body of work on *Anopheles gambiae* s.s. and new theoretical results make it possible to evaluate the likelihood of speciation by ecotypification using a stochastic model. We created a simulation that captures the essential properties of the ecotypification process, validated it against independent theoretical expectations and performed systematized experiments. In addition to testing overall probability that ecotypification may lead to speciation, analysis of the experimental results shows which factors are most important for inversion polymorphism. Our study of this synthetic system is offered as a guide to future field and laboratory investigations of speciation in *An. gambiae* and related taxa.

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FEEDING AND RESTING BEHAVIOR OF *ANOPHELES LONGIPALPIS* (THEOBALD) IN AN AREA OF HYPERENDEMIC MALARIA TRANSMISSION IN SOUTHERN ZAMBIA

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Anopheles longipalpis (Theobald) (Diptera: Culicidae) is a predominantly zoophilic mosquito that has not yet been implicated in malaria transmission. However, this species was collected indoors with *An. funestus* s.l. in southern Zambia where transmission of *P. falciparum* is hyperendemic, was confused morphologically and molecularly with *An. funestus* s.l., and has been reported to feed on humans. Our objective was to define the actual or potential role of *An. longipalpis* in transmission of *P. falciparum* in Mufwafwi village in southern Zambia where large numbers of indoor resting, engorged specimens were collected. The resting density, blood feeding behavior and human biting rate of *An. longipalpis* was investigated during the 2004-2005 and 2005-2006 transmission seasons. Numbers of *An. longipalpis* peaked towards the end of the rainy season.

Although 6 specimens were collected during human landing catches, the feeding behavior of this species was significantly biased towards cattle (88.7%), with other blood meals originating from dogs (7.6%) and goats (3.8%). Zero specimens of *An. longipalpis* were infected with *P. falciparum* based on PCR assays. Therefore, *An. longipalpis* was not confirmed to be involved in malaria transmission, although more extensive screening is needed. Correct morphological and molecular identification of this species is crucial for malaria control programs in areas where *An. funestus* s.l. and *An. longipalpis* exist sympatrically so that scarce resources are not wasted on the control of an apparent non-vector.

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INCREASED DETECTION RATE OF LEPROSY (HANSEN'S DISEASE) AND STRATEGY FOR DISEASE CONTROL IN RIO GRANDE DO NORTE, BRAZIL

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Hansen's disease (HD) remains an important public health problem in Brazil, where 38,410 new cases were detected in 2005, with the prevalence rate of 1.48 cases for 10,000 people, larger than the incidence recommended by the World Health Organization for elimination of the disease. HD has a focal distribution in Brazil and the prevalence varies by region. The Northeast Region has the third largest new case detection rate (NCDR) in the country (3.07 new cases per 10,000 people); however the state of Rio Grande do Norte State has one of the lowest NCDR in the Northeast. Careful analysis on reportable data between 1996 and 2005 showed that NCDR has rapidly increased; from 0.64 in 1996 to 1.60 new cases per 10,000 people in 2005. We present here data from a case-control study conducted in Rio Grande do Norte, Brazil. Geographic mapping of the HD cases, residential census, examination of HD cases and household contacts and genotyping of *Mycobacterium leprae* in Rio Grande do Norte were performed. A total of 258 households were visited with 724 of the 808 (89.6%) contacted subjects giving consent to participate in the study. Fifty seven of 724 (7.9%) people examined had skin or nerve findings suspicious for HD. Fifteen new cases of HD were confirmed, giving a detection rate of 2 new cases per 100 people examined. New HD cases were diagnosed by clinical and bacillary findings according to the Ridley-Jopling classification. All cases were started on WHO multi-drug treatment regimens for HD. The georeference of the HD cases using GPS showed that 76.8% of the cases detected lived in areas where the demographic density was high. The results of the case-finding activities show evidence for continued attention to HD case finding and the need to start early treatment. The observation of families with multi HD cases shows the need for studies to clarify the role of human genetic susceptibility and immune responses to *M. leprae* in determining progression to disease development.

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THE RISK OF LEPROSY IN INDIVIDUALS WITH A LOW AND HIGH HOUSEHOLD SOCIO-ECONOMIC STATUS IN NORTHERN BANGLADESH

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Population studies show that an increase of socio-economic circumstances leads to a decrease of leprosy incidence. The relation between socio-economic status (SES) and leprosy at an individual level is uncertain. This study was conducted to determine the risk of leprosy for individuals with a high and low household SES. We conducted a case-control study in Bangladesh. Fifty leprosy cases were sampled from the study population of a prospective study on household contacts, and 100 controls were sampled from a multi-stage cluster sample of the general population. The household SES of cases and controls was determined using an asset index. Principal components analysis was used to determine weights for the individual assets. The relation between household SES and leprosy prevalence was determined through logistic regression modelling. The odds ratio between households with a low SES and a high SES was 3.0 (95% CI 1.33 - 6.79), indicating a three-fold increased risk of leprosy in poor households compared to rich households. In conclusion, the asset index is a practical tool to describe the SES of households. The increased prevalence of leprosy in households with a low SES may possibly be used to target control activities more effectively.

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COLEP: A CLUSTER RANDOMISED CONTROLLED TRIAL WITH SINGLE DOSE OF RIFAMPICIN TO PREVENT LEPROSY AMONG CLOSE CONTACTS OF NEWLY DIAGNOSED LEPROSY PATIENTS IN BANGLADESH

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Close contacts of leprosy patients are at an increased risk of developing clinical leprosy, the disease caused by infection with ***Mycobacterium leprae***. In the past it was shown that prophylaxis with dapsone could prevent leprosy among contacts, but this had to be administered over a long period of time. Rifampicin is a strong bactericidal drug against ***M. leprae*** and more effective than dapsone. Uncontrolled or unblinded studies have shown that it is effective when used as a prophylactic drug against leprosy.

We performed a single-centre, double blind, cluster-randomised, placebo-controlled trial among 21,711 close contacts of 1037 newly diagnosed leprosy patients in northern Bangladesh. The intervention consisted of a single dose of rifampicin prophylaxis or placebo given to all contacts in the second month after the treatment of the patient had started. 19,956 contacts (91.2%) were examined for leprosy during the first follow-up after two years. The reduction in incidence after two years by a single dose of rifampicin was nearly 50% and the number needed to treat to prevent a single case of leprosy among contacts was 287. During the presentation the trial, its outcomes and further details with regard to specific risk groups will be presented. It will be discussed whether rifampicin prophylaxis is useful additional tool in leprosy control, which contacts would benefit most and operational issues which need to be taken into account when introducing prophylaxis in field programs.

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PREVENTION OF LEPROSY USING RIFAMPICIN AS CHEMOPROPHYLAXIS: RESULTS AFTER 6 YEARS FOLLOW-UP

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In 2000 a controlled chemoprophylactic intervention study started on five Indonesian island highly endemic for leprosy (4739 inhabitants in total) to determine whether rifampicin can be used as chemoprophylaxis to prevent leprosy. The population was actively screened before the intervention and subsequently once yearly for 6 years. On the control island no

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chemoprophylaxis was given. On the largest island chemoprophylaxis was only given to household and neighbour contacts of leprosy patients ('contact' group) and on three small islands to all eligible persons ('blanket' group). The prophylactic regimen consisted of two times 600 mg rifampicin for adults and 300 mg for children (5-14 years) with four months between doses. At all islands the leprosy patients received regular MDT treatment.

The total cohort consisted of 3,964 persons, initially free of leprosy. After three years follow-up the yearly incidence rate in the control group was 39/10,000; the cumulative incidence was significantly lower in the blanket group (p=0.031). No difference was found between the contact and the control groups (p=0.93). To see whether this apparent reduced leprosy incidence in the first three years in the blanket group was due to a delayed development of leprosy or a complete clearance of infection the cohort was followed for another three years. The initial lowering effect of the chemoprophylaxis in the blanket group on the incidence of leprosy in the first three years was not seen in the second three years of the study; data analysis is currently ongoing. More results will be presented during the congress.

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ISOLATION AND CHARACTERIZATION OF *BARTONELLA BACILLIFORMIS* FROM AN EXPATRIATE ECUADORIAN

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An atypical *Bartonella bacilliformis* infection was diagnosed in an expatriate patient with chronic keloid-like skin lesions and splenomegaly three years after visiting Ecuador. Initial serology, PCR, and immunohistochemistry ruled out *B. henselae* and *B. quintana*, but pathology of the splenic biopsy was suggestive of bacillary angiomatosis. Bacilli subsequently isolated from the patient's blood were identified as *B. bacilliformis.*EC01 was isolated from blood after 18 days incubation on 5% rabbit blood-heart infusion agar with phosphate buffer overlay at 28°C. DNA was analyzed by nested PCR using a panel of *Bartonella* gene primers, protein banding patterns were determined by 1-D gel electrophoresis and Western blot, and EC01 antigens were tested by immunofluorescence assay (IFA) against a panel of antisera from patients with Oroya fever in Peru. This case was unusual as the patient was inapparently infected with *B. bacilliformis* in a nonendemic area of Ecuador and clinical signs of illness were greatly delayed and atypical for classical bartonellosis. The EC01 gene sequences (*gltA* and ITS) and protein band banding pattern were most similar to a sub-set of *B. bacilliformis* isolates from the endemic region of Caraz, Ancash in Peru. By IFA, patient serum did not react with all Peruvian *Bb* isolates tested nor did EC01 antigen react with all Oroya fever sera. Hyperimmune rabbit antisera to *B. bacilliformis* bound to similar proteins of EC01 and other isolates of *B. bacilliformis* from Peru by Western blot. Electron microscopy also showed the presence of peritrichous flagella on EC01. In conclusion, infections with *B. bacilliformis* may present with unusual clinical signs and be missed by standard diagnostic tests, so it is important to attempt isolation. The true distribution of *B. bacilliformis* in South America, as well as patient and microbial factors which contribute to atypical symptoms of infection, are poorly understood.

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IMMUNOLOGICAL PATTERN OF PATIENTS WITH ACUTE AND CHRONIC PHASE OF *BARTONELLA BACILLIFORMIS* INFECTION IN A ENDEMIC AREA IN PERU

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Human Bartonellosis is produced by *Bartonella bacilliformis*, a flagellated gram negative bacterium, and may have two clinical presentations. The acute phase is characterized by a feature of sepsis with RBC's parasitemia, or a chronic phase characterized by cutaneous lesions. This is the first study that evaluates the immunology patterns of patients in endemic area. It was a transversal pilot study that included patients in two hospitals in Ancash-Peru, and one hospital in Lima-Peru. Patients between 5 and 60 year-old, with acute or chronic phase according standardized Peruvian criteria were included. Pregnancy, previous immunosuppression, or recently antibiotics use were exclusion criteria. T CD4+, and CD8+ cells recount cells was done by FACSCalibur flux cytometer. IFN-γ, IL-2, TNF-ζ, IL-4, IL-10 measured were by BD OptEIA™ ELISA Kits. Data were analyzed using SPSS 11v. Variables were transformed to Log10 for ANOVA one-way and Tukey Pairwise comparisons. With previous consent, 9 people in the control group, 21 patients with acute phase, and 17 with chronic phase were included. The ANOVA-one way analysis showed differences among groups for INF- γ ($p<0.005$) and IL-10 ($p<0.005$). In the acute phase, the mean of CD4+ was 866.5, CD8+ 829.5, INF- γ 152.34, IL-10 99.39 (SD 121.45). In chronic phase, the mean of CD4+ was 808.12, CD8+ 714.94, INF- γ 23.69, IL-10 23.36 (SD 27.99). In Tukey analysis, for INF-γ and IL-10, there were statistic difference between the acute and chronic phase group ($p<0.001$), and between the acute and control groups ($p<0.001$) only for IL-10. Using binomial probability, in the acute phase, there was a significant difference between expected and observed CD4+ ($p<0.001$) and CD8+ outliers ($p<0.001$), with 6/10 and 5/6 outliers over normal range respectively; also, in the chronic phase in CD4+ ($p<0.005$) and CD8+ ($p<0.001$), with all outliers over normal range. In conclusion, CD4+ and CD8+ T cells recount were abnormal in acute and chronic phase, mainly over normal limits, however, some patients during the acute phase had values below normal range, that may explain infectious complications. Significant high level of IL-10 was found in acute phase. In gram negative sepsis, an uncontrolled production of IL-10 may produce an "immunological paralysis" of antigen presenting cells. This phenomenon may explanation severe courses in some patients, and deserves further study.

(ACMCIP Abstract)

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THE IDENTIFICATION OF *IN VIVO* INDUCED PROTEIN ANTIGENS DURING *BACILLUS ANTHRACIS* INFECTION

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In vivo induced antigen technology (IVIAT) identifies immunogenic bacterial genes expressed specifically during infection and not during *in*

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vitro culturing. Convalescent serum from patients or animals infected with a pathogen of interest are pooled and extensively adsorbed against the cognate pathogen grown under standard laboratory culture conditions. This adsorption step removes antibodies that bind antigens expressed during *in vitro* growth, while retaining antibodies that recognize antigens specifically expressed during infection. Adsorbed serum is then used to probe a protein expression library. We applied IVIAT to *Bacillus anthracis*, the cause of anthrax. We pooled convalescent sera from immunized macaques surviving inhalational challenge with fully virulent Ames strain *B. anthracis* spores. We adsorbed this pooled sera against *B. anthracis* organisms grown in BHI broth in air. We then used the adsorbed serum to probe an inducible 125,000 clone *B. anthracis* protein expression library established in *Escherichia coli.* We identified twenty *B. anthracis* antigens as immuno-reactive using IVIAT, including PagA (protective antigen; supporting the validity of the screen), and six members of a N-acetylmuramoyl-L-alanine amidase (NALAA) family. Using quantitative real time PCR comparing RNA isolated from *in vitro* cultured cells to RNA isolated from BALB/c mice infected with virulent Ames strain, we confirmed induced expression *in vivo* for a subset of *B. anthracis* genes identified by IVIAT, including *pagA*, NALAAs *amiA* (pXO2-42), BA3767 and *plyL* (BA4073), and a putative bacteriophage holin gene, BA4074 (possibly co-transcribed with BA4073). We have generated histidine-tagged fusion proteins for two *B. anthracis* antigens identified by IVIAT, and confirmed a specific immune response post-wild-type *B. anthracis* challenge in a subset of macaques for NALAA BA3737. The identification of immunogenic *B. anthracis* proteins expressed *in vivo* during anthrax could have diagnostic, therapeutic or preventative implications for this zoonotic infection.

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THE IMPACT OF SCHISTOSOMIASIS AND INTESTINAL HELMINTH CONTROL PROGRAM ON HEALTH IN RURAL UGANDA

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Two rounds of treatment with praziquantel and albendazole have been delivered in Uganda to high risk communities, and monitored annually by following a cohort of children before anf after treatments. 1871 children were successfully examined on three annual visits. There was evidence that mass treatment with praziquantel and albendazole lead to a significant reduction in intensity of *Schistosoma mansoni* (70% (95% Confidence Interval [CI] 66-73%) after one year and 82% (95% CI: 80-85%) after two years). Intensity of hookworm infection was also reduced (75% and 93% - unadjusted). There was also a significant increase in haemoglobin concentration (0.135 g/dL (95% CI: 0.126-0.144). after one year and 0.303 g/dL (95% CI: 0.293-0.312) after two years), and significant reductions in signs of early clinical morbidity. The impact of intervention on *S. mansoni* levels was similar to that predicted by mathematical models of the impact of chemotherapy for human schistosomiasis. Improvements in haemoglobin were greatest for those children found to be anaemic or harbouring a heavy *S. mansoni* infection at baseline. In conclusion, anthelmintic treatment delivered as part of a national helminth control programme can reduce infection and morbidity due to schistosomiasis and intestinal helminths and can improve haemoglobin concentrations of schoolchildren.

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MONITORING URINARY SCHISTOSOMIASIS INFECTION IN COMMUNITIES GIVE A PRAZIQUANTEL 'HOLIDAY' AFTER FIVE ROUNDS OF TREATMENT

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Mass drug distribution of praziquantel (PZQ) at a dose of 40 mg/kg every 1-3 years can significantly reduce schistosomiasis morbidity in areas of high endemicity. However, the optimal interval for such treatment is debated. PZQ, which is not donated by pharmaceutical companies to National Control Programs, costs about US $0.08 per 600 mg tablet. Therefore, drug costs in mass treatment programs can become substantial, and economizing through drug PZQ 'holidays' could allow more people to be treated by programs using drug rotation schemes through different endemic areas. In the local government area (LGA) of Pankshin in Plateau State, Nigeria, PZQ treatment for urinary schistosomiasis was launched in 1999. PZQ was administered in schools in communities where a sample of 30 children aged 10-14 were found to have a hematuria prevalence by dipstick of ≥20%-<50%. In communities with higher prevalence (≥50% hematuria) PZQ was administered community wide (e.g., including adults). In eight sentinel villages in the LGA (4 receiving school-based treatment and 4 receiving community-wide treatment) we observed a dramatic decline in hematuria over a four-year period from a baseline mean of 40% (village range 30%-77%) hematuria prevalence among 240 children in 1999 compared to a mean of 5% (range 0-27%) among a independent group of 240 just prior to the fifth PZQ treatment administration in 2003. In consultation with the ministry of health of Nigeria, PZQ treatments were stopped after the fifth dose, while simultaneously intensifying a schistosomiasis health education campaign throughout the LGA. Two years after stopping PZQ mass treatments, in 2005, we again evaluated 240 children in the eight sentinel villages to look for evidence of recrudescence. We found the 2% hematuria rate (range 0-7%) to be essentially unchanged from 2003. We concluded that recrudescence had not occurred after a 2 year 'drug holiday' interval and a three year rotation would be 'safe.'

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HELMINTH INFECTIONS AND POLYPARASITISM AS PREDICTORS OF COGNITIVE PERFORMANCE OVER 18-MONTHS OF FOLLOW-UP AMONG SCHOOL-AGE CHILDREN

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The objective of this study was to estimate the effect of *Schistosoma japonicum* reinfection and changes in the burden of soil transmitted helminth (STH) infections on cognitive test performance. Longitudinal treatment-reinfection study with treatment for *S. japonicum* infection. Four tests were administered including: learning and memory domains of the wide range assessment of learning and memory (WRAML), verbal fluency and the Philippine nonverbal intelligence test (PNIT). Infection burden were determined for STH and *S. japonicum* and used to define infection intensity based. A dichotomous variable denoting: low reinfection burden vs. no change/higher reinfection, was defined for each species. Repeated measures multiple regression analysis was conducted

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to assess the impact of changes in infections on test scores. None/low *S. japonicum* reinfection was predictive of high scores in the PNIT (=1.05, p-value=0.016) and WRAML learning (=2.33, p-value=0.014). Ascaris (=4.10, p-value=0.028) and trichuris (=4.17, p-value=0.016) intensity reduction were predictive of higher longitudinal performance in WRAML learning and WRAML memory respectively. Baseline number of STH infections was associated with lower scores in WRAML learning (= -2.49, p-value=0.052) and PNIT (=1.03, p-value=0.058) and decline in polyparasitic STH infections was predictive of higher performance in WRAML learning for girls (=4.13, p-value=0.023). In conclusion, low *S. japonicum* reinfection, longitudinal decline in ascaris and trichuris intensity, and reduction of polyparasitic STH infections were predictive of higher performance in 3 of 4 cognitive tests suggesting that simultaneous control of *S. japonicum* and STH infections could improve children's ability to take advantage of educational opportunities in helminth endemic regions.

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MODELING SCHISTOSOMIASIS TRANSMISSION IN A DISTRIBUTED ENVIRONMENT: IMPLICATIONS FOR SUSTAINABLE CONTROL

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Social interaction and physical interconnections between villages can influence the spread of infectious agents. Using a mathematical model of schistosomiasis transmission for a distributed set of heterogeneous villages, we explored the effect of two forms of connectivity on disease transmission and control. One form of connectivity, cercarial and miracidial transport, occurs via hydrological channels that link adjacent villages. Another form of connectivity, social behavior, such as migrant labor, can lead to infection of laborers outside of their own villages, and potentially the spread parasite eggs to other villages. We modeled 15 hypothetical connected villages, each with differing potential to sustain transmission. We show that the two forms of connectivity can have important consequences for disease control. First, transmission can be sustained regionally throughout a group of connected villages even when individual village conditions would appear to not support endemicity (Basic Reproductive Numbers for all individual villages are less than one). Second, certain levels of connectivity lead to optimum transmission, which somewhat surprisingly, does not necessarily coincide with the largest spread of social contacts. Third, the targeting of villages with high snail and human infection, without regard to village interconnections may not lead to sustainable control. Analyses of distributed models may provide valuable insight into more sustainable control for a variety of parasitic diseases.

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SOCIO-ECOLOGY OF MALARIA AND URINARY SCHISTOSOMIASIS IN COASTAL KENYA

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Schistosomiasis and malaria are "diseases of poverty" and are co-endemic in many parts of the world. Despite the high global prevalence of these diseases few studies have examined the potential effects of co-infection on morbidity. Additionally, these diseases may share common upstream socio-economic or environmental risk factors. An improved understanding of the socioeconomic and ecologic context for such multiple infections may help focus prevention and control efforts. Accordingly, we undertook a study to examine the contextual determinants of polyparasitism in coastal Kenya, with a focus on *Plasmodium* and *Schistosoma* infections. Following informed consent, 1300 eligible participants age 8 and above in Kingwede, Kenya were recruited to participate during April and May, 2006. Presence and intensity of *S. haematobium* infection was determined using standard urine filtration examination. Presence and intensity of *P. falciparum* infection was determined using standard thick and thin blood slide examination as well as by PCR. Hemoglobin, height and weight measures were also taken for children to determine anemia and stunting (via Z-scores). Participants were administered a detailed questionnaire in Kiswahili addressing schistosomiasis and malaria knowledge, self-reported water use and other health practices, as well as socioeconomic status (SES). The importance of these factors was assessed using regression models compared to marginal models that incorporate correlation of individuals within households. We also examined spatial patterns of schistosomiasis and malaria cases, determining environmental risk factors based on cluster analyses. Standard and multilevel analyses revealed important household level socio-economic risk profiles related to health knowledge and behaviors (e.g. water contact patterns, use of treated bed nets) that appeared to affect risk.

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TOWARDS INTEGRATED CONTROL TO ACHIEVE TERMINATION OF SCHISTOSOMIASIS TRANSMISSION IN IRRIGATED AGRICULTURAL REGIONS OF CHINA

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In the aftermath of the SARS outbreak in 2003, the Chinese government launched campaigns against a number of major communicable diseases including the parasitic disease schistosomiasis. To beef up the control, the State Council of China recently issued an unusual specific statute on schistosomiasis control calling for a comprehensive approach. Two endemic mountainous provinces with typical irrigated agriculture, Sichuan and Yunnan, were chosen to pilot aggressive control efforts and declared ambitious goals of effectively controlling schistosomiasis by 2008 and eliminating the transmission throughout the provinces by 2015. Here we report on village-level studies in Sichuan of the determinants of transmission and efforts required to effectively control even eliminate the disease transmission using a mathematical model. The model specifically incorporates time-varying environmental variables (e.g. water contact and temperature- and rainfall-driven events) which are believed to play important roles governing the transmission cycle. A new metric of transmission potential, environmentally-mediated Ro, or basic reproductive ratio, arose from the study. The model was calibrated to field data and the calibrated model utilized to explore a range of competing control strategies. The results offer evidence of inadequacy of the niclosamide-praziquantel (molluscicide)-chemotherapy) strategy to achieve broadly sustainable intervention of transmission in this environment. It suggests that the goal of eliminating transmission will require environmental modifications and/or improved sanitation facilities, for example, alternation of village systems to permanently destroy snail habitat or better waste management to control parasite eggs, which permanently change the disease transmission potential, in addition to the continued use of paraziquantel and niclosamide. The study has important policy implications for the ongoing schistosomiasis control in China.

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SPATIAL DISTRIBUTION OF URINARY SCHISTOSOMIASIS INFECTION AMONG SCHOOL CHILDREN IN AN ENDEMIC COMMUNITY IN GHANA

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Schistosoma haematobium infection is one of the major contributors to the disease burden in Ghana, after malaria. Careful detection of high transmission epicenters may offer the potential for a more effective, highly-focal snail control in conjunction with targeted chemotherapy to reduce transmission. In the present study, the Geographic Information System (GIS) was applied to incorporate demographic, parasitological, and household location data for school children in an endemic village, in the Cape Coast Municipality in Ghana. The prevalence of infection was 32.1% with the highest infection recorded among children in the age group 12-14. More males (33.7%) were infected compared to females (29.4%) and this is likely a result of differences in social and religious practices. However, there was no evidence of significance between them (^{2} = 0.142, P = 0.71, CI = 95%). A strong positive correlation was found between water contact activity and infection (^{2} = 27.164, P < 0.001, CI = 95%). GIS techniques were utilized for producing maps and analyzing the results. High infection intensities were clustered in the community and around different water contact sites. Though these results are preliminary, it confirms the small-scale focalization of the disease and shows that the GIS can be an important tool for schistosomiasis control, especially in areas where the introduction of alternative water sources and the implementation of mass community chemotherapy have failed to halt the continuing cycle of urinary schistosomiasis transmission.

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DISTRIBUTION OF FREE BEDNETS BUNDLED WITH INSECTICIDE VIA AN INTEGRATED CHILD HEALTH CAMPAIGN --- LINDI REGION, TANZANIA, 2005

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Use of insecticide-treated bednets (ITNs), a proven intervention to prevent malaria mortality, continues to be low, and new distribution strategies are needed. From July 30 to August 1, 2005, the Tanzanian Ministry of Health and UNICEF conducted an integrated health campaign with free distribution of untreated bednets bundled with insecticide, measles vaccination, vitamin A and mebendazole for children <5 years old (under-5s) in Lindi Region, Tanzania. Only written and illustrated instructions on treatment and use of bednets were provided. A community-based cross-sectional cluster survey to assess intervention coverage was conducted from November 2-16, 2005 during low-intensity malaria transmission season. Thirty enumeration areas (EAs) were selected using probability proportional to estimated size. Households in each EA were mapped, and twenty households per EA were randomly selected. Altogether, 574 households with 354 under-5s were visited. Most under-5s (79.6%) received a bednet. Because of the campaign, household possession of any bednet increased from 52.9% to 69.3% (p <0.001) and possession of an ITN increased from 13.3% to 24.7% (p <0.001). The distribution was equitable, and possession of bednets and ITNs increased in all wealth quintiles. Among households that had received at least one bednet, 99.4% reported retaining all campaign bednets. Caretakers reported that 46.3% of under-5s slept under a bednet, and 21.5% of under-5s slept under an ITN the previous night. The total cost per bednet distributed was $2.47 ($2.18 per bednet with insecticide and $0.29 for distribution). Integrating malaria prevention activities with immunization campaigns can rapidly and equitably increase possession and use of bednets and merits continued large-scale implementation. Rates of home treatment of bednets with insecticide were low, thus future distribution campaigns should provide factory-treated long-lasting ITNs. Use of ITNs by under-5s was low, and further work is needed to increase ITN use after distribution campaigns.

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DISTINCT *P. VIVAX* POPULATIONS IN MEXICO DIFFERENTIALLY INFECT TWO LOCAL VECTORS

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Two circumsporozoite (*CS*) protein repeat types (VK210/VK247) have been observed in *Plasmodium vivax* in southern Mexico, and their distribution approximates that of the two local vectors - *Anopheles pseudopunctipennis* and *An. albimanus.* Additionally, they have been shown to be associated with differential infectivity of the two vectors. However, the destruction of VK247 parasites in *An. albimanus* occurs prior to the expression of the *CS* protein. To further investigate the molecular basis of this host-specificity, and to better characterize the parasite population, we genotyped *P. vivax* isolates using 29 genome-wide microsatellites and 23 minisatellites flanking the *csp* gene and spanning 200 Kb. Using a model-based structuring method, we found support for multiple parasite sub-populations that in turn formed two distinct groups, which were supported by bootstrap analysis, showed little evidence of recombination, and differentially infected *An. albimanus* and *An. pseudopunctipennis*. The VK210 repeat type was found in both populations. The *csp* locus showed strong linkage disequilibrium in the *An. pseudopunctipennis* parasite population but not the *An. albimanus* parasite population, leaving open the possibility that one or more genes at this locus contribute to the infectivity phenotype in *An. pseudopunctipennis*.

(ACMCIP Abstract)

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STRAIN- AND SPECIES-SPECIFIC COMPARISON OF THE IMMUNE RESPONSES OF DIFFERENT MEMBERS OF THE *ANOPHELES GAMBIAE* COMPLEX TO *PLASMODIUM FALCIPARUM* INFECTION

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Previous research has indicated that the susceptibility of *Anopheles gambiae* to *Plasmodium* infection varies according to the strain of mosquito and species of malaria parasite, indicating the existence and importance of strain- and species-specific variation in the outcome of mosquito infection with different malaria parasites. However, most laboratory studies are undertaken using only one, or several, mosquito strains and a single malaria species (usually the rodent malaria *P. berghei*) with the assumption that the results are generalizable to other mosquito-malaria parasite combinations. Consequently, we have undertaken global transcriptomic comparisons of naïve and *P. falciparum*-infected adult female mosquitoes from different strains and species within the *An. gambiae* complex using whole genome oligonucleotide microarrays. We have identified a number of constitutive and *P. falciparum*-induced differences in gene expression between the different strains and species within the *An. gambiae* complex which we have functionally characterized using RNAi screens, and compared to *P. berghei* infection in the same mosquito strains/species. Although some aspects of the mosquito response to *Plasmodium* infection were conserved across mosquito-malaria

parasite combinations, our findings highlight the importance of strain- and species-specific differences in the immune responses of different members of the *An. gambiae* complex to infection with different *Plasmodium* species.

(ACMCIP Abstract)

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DRY SEASON MALARIA TRANSMISSION IN A RURAL SUDAN SAVANA OF MALI

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Malaria transmission is considerably reduced during the dry season. In the village of Bancoumana, while adult mosquitoes were barely detectable, studies have shown that new *Plasmodium falciparum* infection occurred during the dry season. The aim of this study was to identify potential sources of *P. falciparum* infection during the dry season and provide basis for selective control strategy in Mali. From December 2004 to May 2005, we carried out a monthly active search of breeding sites and adult mosquitoes collection in Bancoumana village and in a 5 kilometer distant fishing hamlet (Bozokin) lying along the Niger River. Mosquitoes were collected by pyrethrum spray catch methods. Vector infection rate and molecular forms were determinant by ELISA and PCR methods respectively. Passive surveillance was performed to record all microscopically confirmed cases of malaria in children of 0_15 years seeking for treatment at the health center of Bancoumana. Among *Anopheles gambiae complex*, *An. arabiensis* represented 11.4% (17/149) in Bancoumana and only 2.1% (8/374) in the hamlet. The M molecular form of *An. gambiae s.s.* was the most abundant in both localities. However its frequency in Bancoumana (92.6%, n = 95) was significantly higher (X2 = 4.51, P = 0.034) than in the fisher hamlet (84.0%, n = 319). The mosquito monthly biting rates were 0.63 (Minimum = 0.04, Maximum = 1.3) in Bancoumana and 35.4 (Minimum = 15.4, Maximum = 82.1) in the hamlet. The cumulated entomological inoculation rate over the six months of study was only 0.018 infective bites in Bancoumana while it was up to 24.4 infective bites in the hamlet. About 55 cases of fever recorded at the health clinic between March and May; 38% had blood smear positive. All malarial fever cases were resident of Bancoumana (38.2% (21/55% of cases/total number of fever recorded March and May) and none from the Hamlet. In conclusion, the low level of malaria transmission in Bancoumana may be sustained by a high prevalence of infected mosquitoes in the Hamlet where numerous breeding sites were also found as result of drying river. A larval control strategy should target permanent water bodies during the dry season in those areas with seasonal malaria transmission.

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IN SEARCH OF ENVIRONMENTAL DETERMINANTS FOR MALARIA TRANSMISSIONS IN INDONESIA

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With a population of 242 million, Indonesia is the fourth most populous nation in the world. It also has the third highest malaria endemicity in Southeast Asia after Myanmar and India. Approximately 40% of its population lives in malarious regions. The distribution of malaria in Indonesia is highly heterogeneous. On Java and Bali, the two islands where about 70% of the population concentrates, malaria is hypoendemic. But on the Outer Islands, which include the rest of the archipelago, malaria ranges from hypo- to hyperendemic. The Indonesian archipelago spans approximately 5,000 km. Within this wide geographic area, a number of dominant anopheles species are responsible for the major part of malaria transmissions. How the larval habitats of these species are affected by and respond to climatic, environmental, and human-induced changes are reflected in seasonal and longer-term variations in malaria transmissions. Understanding the ecology of these species also points to the way of using environmental management to reduce the propagation of malaria vectors. Passive and active case detection data from previous years for a number of Indonesian provinces are analyzed. Environmental parameters used in the study are extracted from surface observed and satellite measured datasets, including NASA's Tropical Rainfall Measurement Mission, Moderate Resolution Imaging Spectroradiometer, and Germany's Global Precipitation Climatology Center data. Using the neural network method, an artificial intelligence technique, we have characterized the relationship between malaria transmission and environmental parameters. The results may also be used for early warning malaria epidemics and outbreaks.

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EPIDEMIOLOGICAL AND CLINICAL CHARACTERISTICS OF THE REEMERGING VIVAX MALARIA IN THE REPUBLIC OF KOREA

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Vivax malaria, which was prevalent in the Republic of Korea (= South Korea), disappeared rapidly since the 1970s, but re-emerged in 1993 near the demilitarized zone (DMZ), the border between South and North Korea. After the first reemergence, malaria prevalence increased exponentially, peaking in 2000, and then decreased; in total, 21,390 cases were reported between 1993 and 2005 in South Korea. The major infection source is, even at present, mosquitoes infected in North Korea and flying across the DMZ. Thus, this reemerging malaria is regarded as a peculiar type of border malaria; only mosquito vectors can come and go. During 1993-1997, the majority of cases (81.2%) were soldiers and veterans who worked in the northern parts of South Korea. However, during 2002-2005, soldier and veteran cases decreased (46.4%), and civilian cases (presumably local transmission) increased. The main transmission season is June to September each year; during this season vector mosquitoes *Anopheles sinensis*-complex, which includes *An. pullus* and *An. lesteri*, are very active. The reemerging malaria patients characteristically reveal combination of short (1-2 months) and long incubation periods (5-13 months) with predominance of the long type (2/3 of patients). Fever intervals are usually 48 hours, but frequently (20% of patients) atypical. Anemia is not commonly encountered, but thrombocytopenia is common. There are a few subclinical cases with apparent parasitemia. Various control measures have been operated, which must have greatly influenced the reduction of incidences.

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CHANGE IN MALARIAL PARASITEMIA PREVALENCE AND INDOOR RESIDUAL SPRAYING: EVIDENCE OF A DOSE RESPONSE RELATIONSHIP

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The Bioko Island Malaria Control Project (BIMCP) initiated a comprehensive malaria control intervention since February 2004, funded by a consortium led by Marathon Oil Company in partnership with the government of Equatorial Guinea (EG). The measures consist of indoor residual spraying (IRS) and case management including effective treatment based on combination therapy. Under the BIMCP all houses were sprayed once with pyrethroid insecticide in 2004; from January 2005 houses were sprayed with carbamates at 6 monthly intervals. Annual parasitemia household surveys were conducted in March 2004, 2005 and 2006 respectively

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at 18 sentinel sites. A total of >10,000 children were tested in the three surveys. Household data included information on spraying, house construction, bednet use, illness histories and indicators of household wealth. Average prevalence of infection with *Plasmodium falciparum* at all sites combined reduced in all age groups 2 to <15 years from 45% at baseline to 31% in 2005 (p<0.001) and 26% in 2006 (p<0.03), with substantial between site variation. Reported site specific spray coverage of houses in the 2006 survey ranged from 58% to 87%. Odds of infection was significantly lower for children living in houses that had been sprayed (OR=0.67 relative to unsprayed houses, p<0.001), regardless of whether the child slept under a bednet or not. The odds of infection of an individual child decreased by a factor of 0.94 (p=0.035) for every one percent increase in spray coverage of the neighbourhood in which the child lived, independent of the spray status of her/his own home. Desire to have houses sprayed was uniformly high (mean 92%). Substantial overall reduction in prevalence of infection with *P. falciparum* in children can be achieved in equatorial settings provided a high level of spray coverage is maintained. Risk of infection is simultaneously related to the spray status of the child's home and to the spray-coverage achieved in the neighbourhood of the house.

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USING TREATMENT FAILURE TO SCREEN FOR MDR TB IS ASSOCIATED WITH RECURRENCE, DEATH AND TRANSMISSION

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In many resource-poor settings, TB drug susceptibility testing for all newly-diagnosed patients using traditional methods is not feasible. Instead, patients are given a trial of first-line drugs, and those who fail are then tested for MDR TB. This strategy seems to reduce both diagnostic and treatment costs because many MDR TB patients appear cured by first-line agents. No data exists, however, concerning the long term outcome of MDR TB patients "cured" by first-line drugs. 351 patients from a community hospital in Lima, Peru were enrolled after new diagnoses of TB disease. Patients were tested for resistance to rifampicin and isoniazid, followed throughout treatment, and interviewed a median of 60 months after treatment. This long term follow up established TB-related morbidity and mortality in both index cases and contacts. Cases of recurrence or contact TB were confirmed with health post records. Despite laboratory test results reporting 21 cases of MDR TB to patients and physicians, all 351 enrolled patients received a complete trial of first-line agents. Twelve of 21 patients(57%) with laboratory confirmed MDR TB converted to sputum smear negativity and were considered "cured" by first-line drugs alone. At long term follow up, however, patients cured of MDR TB were more likely to suffer recurrence (HR=18, 95%CI=7-45, p<0.001) and to die of TB (HR=7, 95%CI=1.4-38, p=0.018) than patients cured of non-MDR TB in Cox proportional hazard regressions accounting for differences in HIV prevalence and other risk factors for new TB infection. Among MDR TB patients, "cure" was not associated with a statistically significant reduction in long-term mortality (p=0.3). In addition, contacts of "cured" MDR TB patients suffered four times as many episodes of new TB as contacts of cured non-MDR TB patients during follow up (HR=3.8, 95%CI=2-9, p=0.001). There was similar contact TB incidence between MDR and non-MDR households prior to the the study, implying that delayed MDR TB diagnosis may have caused increased transmission. In conclusion, the majority of MDR TB patients appeared cured by first line agents, but usually this was a false cure, and MDR TB patients treated with first-line drugs were at high risk of TB relapse, death, and transmission. Using treatment failure to identify drug resistance underestimated MDR TB, and the resultant delays in optimal therapy were associated with mortality and MDR TB dissemination.

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HUMAN CELL-MEDIATED IMMUNITY AGAINST *MYCOBACTERIUM TUBERCULOSIS* ANTIGENS IS AUGMENTED BY TREATING INTESTINAL HELMINTHS

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Tuberculosis and intestinal helminth infections frequently co-exist and helminths cause immunosuppression, micronutrient deficiency and anergy. We hypothesized that treating intestinal helminths may augment antimycobacterial immunity. A double-blind, randomized, placebo-controlled trial in 140 healthy adults living in the Peruvian Amazon. Anti-mycobacterial immunity was assessed by measuring the size of cutaneous induration 48 hours after a 5 unit intradermal tuberculin skin test and by quantifying γ-interferon secretion following whole-blood stimulation with the specific *Mycobacterium tuberculosis* antigens ESAT-6 and CFP-10 (the Quantiferon in-the-tube assay). These *in vivo* and *in vitro* tests were performed at recruitment and 4 weeks after treatment with 3 daily doses of placebo or 400mg albendazole. Stools were examined by direct and concentrated quantitative microscopy. A stool examination at recruitment diagnosed intestinal helminths in 48% of 126 participants. 40% were infected with *Ascaris lumbricoides*, 12% *Trichuris trichuria,* 6.3% hookworms, and 3.2% *Strongyloides stercoralis*. Albendazole therapy caused helminth prevalence to fall to 6.9% (4/58) 2 weeks later (P=0.003) and to 15% (7/48) 4 weeks later (P<0.001). Eosinophil counts fell from median 271 cells/mm^3 at recruitment to 201 cells/mm^3 4 weeks after albendazole therapy (P=0.002). At recruitment, 39% (52/135) of Quantiferon assays were positive and this did not change significantly with albendazole therapy. In contrast, 56% (78/139) of participants were tuberculin skin test positive at recruitment and albendazole therapy caused tuberculin skin tests to increase in size compared wth placebo (P=0.03). In conclusion, treating intestinal helminths significantly augmented antimycobacterial immunity *in vivo* over a one month interval. Therefore, the interpretation of tuberculin skin test results may be complicated by antihelminthic treatment. Prevention or treatment of intestinal helminths warrants evaluation as a potential strategy for reducing tuberculosis susceptibility.

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BACTERIAL MENINGITIS AMONG 0- TO 15-YEAR OLD CHILDREN ADMITTED TO A PEDIATRIC REFERRAL CENTER IN BAMAKO, MALI

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Introduction of conjugate polysaccharide vaccines into infant immunization programs has resulted in dramatic declines in bacterial meningitis. However, global uptake of these vaccines has been slow, particularly in resource-poor countries of Africa. Barriers have included cost, few local demonstrations of impact, and lack of data on disease burden. To assist in quantifying the burden of meningitis in Mali, we performed hospital-based surveillance. Children 0-15 years old with fever ≥ 39° C or suspected bacterial meningitis admitted to the major children's hospital in Bamako were eligible. A blood culture was collected from each child, and cerebrospinal fluid (CSF)), obtained at the discretion of the treating physician, was also cultured. Microbiologically confirmed cases of meningitis were defined by the isolation of a pathogen from CSF. From June 2002 to May 2005, 5514 (90.6% of eligibles) were enrolled. Among the 3176 cases of clinically suspected meningitis, 2991 CSF samples were cultured (94%) and 558 yielded a pathogen (19%). Thus ~5% of hospital admissions had confirmed bacterial meningitis. The most common pathogens were *Haemophilus influenzae* type b (Hib; n = 247; 44%), *Streptococcus pneumoniae* (SP, n = 192; 34%) and *Neisseria meningitidis* (NM, n=42; 8%). 59% of SP belong to serotypes 1, 4, 5, 6B, 9V, 14, 18C, 19F, and 23F. Most NM were either type A (69%) or W135 (21%). Mortality was 19% among microbiologically confirmed cases. Adjusted independent risk factors for mortality were infection with SP (odds ratio [OR] 2.4; 95% CI 1.4-3.9), seizures at the time of admission (OR 2.3; 1.4-4.0), CSF WBC greater than 1,000/mm^3 (OR 1.9; 1.1-3.2) and body temperature ≥40 ºC (OR 1.9; 1.1-3.4). Receiving antibiotics in the week prior to admission was protective (OR 0.5; 95% CI 0.3-0.8). In conclusion, the incidence and mortality from bacterial meningitis in Mali is high, and most cases (~80%) are potentially vaccine-preventable. Ongoing introduction of routine Hib vaccine for infants should confer substantial benefits.

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BURDEN OF INVASIVE BACTERIAL INFECTIONS AMONG CHILDREN ADMITTED TO A PEDIATRIC REFERRAL CENTER IN BAMAKO, MALI - 2002 - 2006

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While much of the burden of childhood infection is borne by those in developing countries, most diagnoses are based on clinical findings and are not confirmed by laboratory studies. Due to limited resources, there is little data on the etiology and epidemiology of these infections. Such information is important for physicians who treat children in these settings as well as local decision makers who are seeking prevention methods. A clinical microbiology laboratory was established at the Hôpital Gabriel Touré. Children age 0-15 years with fever ≥ 39° C or syndromes compatible with invasive bacterial disease (e.g. meningitis, pneumonia) and admitted to the hospital were eligible. Blood and relevant body fluid (e.g. cerebrospinal fluid (CSF)) were cultured. Bacteria were identified by standard microbiologic techniques. From June 2002 to May 2005, 6087 of the 11671 children admitted to HGT were eligible and 5514 children (90.6%) were included. Most (4759; 86%) were from Bamako and 2839 (51.5%) were <1-year old. Pathogens were isolated in blood and/or other fluid from 1319 (24%) participants. The most common isolates among those children with fever without localizing signs were Gram negative bacilli (other than *Salmonella spp*) and non-typhoidal *Salmonella* (NTS). Those with suspected meningitis were more likely to have *Haemophilus influenzae* type b (Hib) and those with suspected pneumonia, *Streptococcus pneumoniae*. Among 0- to 11-month olds, 719 (25%) had a positive culture; Hib (n = 300) and *S. pneumoniae* (n = 201) were the most common isolates. Among 1- to 4-year olds, 360 (21%) had a positive culture; NTS (n = 110) and *S. pneumoniae* (n = 107) were most common. Among 5- to 15-year old children, 240 (25%) had pathogens, 66 were *S. pneumoniae* and 50 were NTS. In conclusion, the burden of bacterial invasive infections is high among children admitted to HGT. Vaccines against Hib and *S. pneumoniae* have the potential to greatly reduce this burden. Additional information regarding the source of gram negative bacillary and NTS infections is needed to design appropriate intervention strategies.

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AN EVALUATION OF A RAPID SERODIAGNOSTIC TEST FOR TYPHOID FEVER - AN GIANG, VIETNAM 2005-2006

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Typhoid fever causes over 21 million infections and 200,000 deaths worldwide each year. In Vietnam's Mekong Delta, the annual incidence is 198/100,000. Isolation of *Salmonella enterica* serotype Typhi from blood or bone marrow is the most reliable means of confirming acute typhoid fever. However, culture methods are costly, slow, and often unavailable in endemic areas. We assessed the validity of Tubex® TF, a rapid serodiagnostic assay for *S.* Typhi IgM, at the An Giang Province Hospital in the Mekong Delta. We compared Tubex® TF results to blood and bone marrow culture in hospitalized patients with fever for 3 days, temperature 38.5°C, and clinical suspicion of typhoid fever. We enrolled 123 patients from May 2005 to February 2006. The median age was 13 years (range 3-65); 43% were female. Symptoms included anorexia (91%), diarrhea (69%), nausea (68%), and abdominal pain (60%). Patients reported a median of 8 days (range 3-31) of fever before admission; the median admission temperature was 39 °C (range 38.5-41.0). Blood cultures yielded *S.* Typhi in 45 (37%) patients; Tubex® TF was positive in all 45 and in an additional 62 in whom blood cultures were negative. Bone marrow cultures, performed in eight patients, yielded *S.* Typhi in five; Tubex® TF was positive in these five, including two patients whose blood cultures were negative. Tubex® TF was also positive in two of three patients with negative bone marrow cultures, one of whom had a positive blood culture. When compared to blood and bone marrow culture respectively, the sensitivity of Tubex® TF was 100% and 100%; specificity was 17% and 33%, positive predictive value was 42% and 71%, and negative predictive value was 100% and 100%. In conclusion, in an endemic area, Tubex® TF is highly sensitive but has poor specificity and positive predictive value compared to blood culture. Further comparison of Tubex® TF with bone marrow culture is warranted to more accurately assess the test characteristics before its routine clinical use can be recommended.

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CHARACTERIZATION OF LETHAL CASES OF LEPTOSPIROSIS WITH EMPHASIS OF WEIL´S SYNDROME AND SEVERE PULMONARY HEMORRHAGE SYNDROME, IN THE CITY OF SAO PAULO, BRAZIL

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Severe forms of leptospirosis are usually referred as Weil´s syndrome - a triad of jaundice, hemorrhages and acute renal failure - and severe pulmonary hemorrhage syndrome (SPHS). SPHS is an emerging complication worldwide yet there is no clear explanation why its frequency and distribution is increasing. Recent reports from Nicaragua and Peru emphasize that leptospirosis may present as SPHS lacking jaundice or other typical manifestations of the Weil´s syndrome. In Sao Paulo metropolitan area, Brazil, the incidence of severe leptospirosis ranged from 1.8 to 3.76 per 100,000 while fatality ranged from 11 to 18% in the last ten years. An active system of death notification including any combination of Weil's syndrome and SPHS, was started in 2003 in the city of Sao Paulo. AIM: to characterize the clinical features of lethal cases of leptospirosis in Sao Paulo with special focus on the emergence of SPHS. A cross-sectional study was performed in these death notifications from 2004 to 2006 of confirmed cases of leptospirosis. Lethal case was laboratorial confirmed by serology, culture or immunohistochemistry, or was based on epidemiological and clinicopathological grounds. Case fatality was 15% (42/285) in 2004, 11% (28/262) in 2005 and 11% (18/161) until May 2006. Case confirmation by a combination of serology, culture or immunohistochemistry was possible in 57 cases while 16 were identified based on clinicopathological findings and 15 on clinical and epidemiological grounds. In the period, the most common infecting serovars among cases and lethal cases, as predicted by microagglutination highest titer, were Copenhageni, Icterohaemorrhagiae, and Butembo. From 88 lethal cases, necropsies were performed in 31 cases detecting pulmonary hemorrhages in 24/31(78%) cases, being the cause of death of all the 24 patients, while Weil´s syndrome was documented in 27/31(87%) by a combination of shock, renal failure and jaundice. SPHS and Weil´s syndrome coexisted in 26/31 (84%) cases with fatal outcome. In conclusion, case fatality remains high in patients with severe leptospirosis and most deaths are consequence of SPHS. In contrast to other reports from other regions of the world, SPHS and Weil´s triad usually coexist in patients with lethal outcomes from the city of Sao Paulo.

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CLINICAL AND LABORATORY COMPARISON OF HUMAN INFECTIONS WITH DENGUE, INFLUENZA, OR AVIAN INFLUENZA A (H5N1) VIRUSES IN INDONESIA

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Passive, hospital-based surveillance has been conducted in outpatients and inpatients from pediatric and internal medicine departments in up to 30 geographically balanced sites across the Indonesian archipelago since 2004. Patients who met the criteria of influenza-like illness (fever >37.8°C and sore throat or cough) were enrolled and rapid influenza diagnostic tests were performed on site. Throat and nasal swabs shipped to Jakarta were screened for avian influenza A (H5N1) virus by real-time RT-PCR (rRT-PCR). Those found to be negative were cultured in MDCK cells and then analyzed by indirect immunoflourescence (IFA). H5N1 positive samples were shipped to the WHO Influenza Reference Laboratory at the Centers for Disease Control and Prevention for rRT-PCT, viral isolation and molecular sequencing. From January 2005 to March 2006, 853 influenza infections were confirmed including 27 influenza A (H1N1), 46 (H3N2), 307 influenza B and 28 H5N1 viruses. Retrospective analyses of the clinical and virologic characteristics were conducted on 56 patients diagnosed with influenza virus infection and 59 with dengue infection that were hospitalized at similar times and geographical locations. On average, patients with human influenza virus infections were hospitalized on day 3.4 of illness, H5N1 cases on day 4.9 and dengue infections on day 4. The clinical manifestations of all influenza viruses during the first three days were similar (fever, cough, and sore throat). However, dyspnea and bronchopneumonia were found predominantly in patients with H5N1 three to ten days after disease onset (mean: 4.8 days).The mean leukocyte, platelet and lymphocyte counts of H5N1 patients over the first five days of illness were significantly lower (p 0.05) then values among other influenza A or B viruses. These findings demonstrate that among a pool of patients seeking medical care in Indonesia during the initial forty-eight hours after onset of illness, H5N1 infections were often indistinguishable from human influenza or dengue infections. This may contribute to a delay in diagnosis and treatment of patients with H5N1.

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TRANS-SPLICING OF THE DEN2-NGC GENOME AT A HIGHLY CONSERVED SITE BY A GROUP I INTRON RIBOZYME

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The trans-splicing variant of the *Tetrahymena thermophila* group I intron catalytic RNA, or ribozyme, is a powerful tool for post-transcriptional RNA modification. It can be directed to act upon a specific accessible uracil on its RNA substrate through controllable base-pair interactions. In the trans-splicing reaction, a target RNA is cleaved at a precise point and a new exon from a separate RNA molecule is then covalently joined to the upstream cleavage product to create a new mRNA ready for translation. The nature of the ribozyme and the predictability with which it can be directed makes it a powerful tool for modifying RNA in nearly any cell type without the need for genome-altering gene therapy techniques or dependence on native protein cofactors. We have successfully targeted two different uracil bases on the positive sense strand within the highly conserved region common to all serotypes of dengue with the group I intron. Our ribozymes have demonstrated the ability to specifically cleave these bases and covalently trans-splice a new RNA sequence downstream of the targeted site, allowing for *de novo* gene expression triggered by dengue viral infection of the cell. This approach provides a two-tiered response of dengue genome cleavage and new protein expression to the presence of the dengue genome. By altering the configuration of the RNA interactions during the trans-splicing reaction and measuring activity by real-time RT-PCR, we have been able to improve the efficiency with which the intron catalyzes the reaction at the two target sites without altering the functional sequence within the intron itself. We envision this novel approach of infection-triggered *de novo* gene expression to be of great potential in combating the spread of dengue virus at the insect level.

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ROLE OF STRESS RESPONSE MOLECULES IN DENGUE VIRUS INFECTION IN MOSQUITO AND HUMAN CELLS

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The first step in dengue virus infection is the interaction between the envelope (E) protein, with the cellular receptor on the cell surface. Several molecules have been described as part of dengue virus receptor complex in different cell lines, suggesting that the interaction between dengue virus and its receptor(s) is a complex, multi-step process or dengue virus may require a receptor complex formed by a group of molecules present in different susceptible cells. Our group has identified the participation of two heat shock proteins (HSPs), HSP90 and HSP70 as part of dengue virus receptor complex in human cells. They were found associated with membrane microdomains (lipid rafts), whose integrity is important for dengue virus entry. Since during dengue infection a systemic inflammatory stress response is evident, we specifically aimed to determine the role of stress mediators in dengue virus infection in human and mosquito cell lines. We found that during stress conditions, viral entry in mammalian cells and binding in mosquito cells are increased. Additionally, reactivity of antibodies directed against HSP90 to the gp45, the glycoprotein involved in dengue infection in C6/36 cells, and its relocation during stress suggests that gp45 is a HSP-like molecule. On the other hand, molecules involved in innate immune response such as the toll like receptor 4 (TLR4), are relocated to lipid rafts in response to dengue virus interaction. It is possible that different molecules play important roles in Dengue virus entry and/or cell signaling, priming events that could trigger the cellular response to dengue virus infection.

(ACMCIP Abstract)

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ROLE OF DC-SIGN AND FCGRII IN ANTIBODY DEPENDENT ENHANCEMENT OF DENGUE INFECTION

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We established a flow-based assay system to study antibody-dependent enhancement (ADE) of Dengue virus infection. We have applied this technique to cell lines expressing varied levels of DC-SIGN and different subsets of receptors from the Fcg_II family. There is a negative correlation between the level of DC-SIGN, and a positive correlation with the expression of particular Fc receptors, to the ability of cells to undergo ADE. We have expanded these studies to evaluate ADE in primary human dendritic cells and monocytes, testing several donors with a variety of dengue immune sera. Using blocking antibodies and directed knockdown via siRNA, we can selectively inhibit expression of the Fc receptors and DC-SIGN, either individually or in combination. Early evidence points to opposing roles for the isoforms of FcgRII (A and B) in ADE. Dengue virus requires the presence of FcgRIIA on K562 cells in order for ADE to occur. Cells with high levels of DC-SIGN do not undergo ADE, but in cells with low DC-SIGN, FcgRIIB appears to inhibit ADE. We will report our current data and progress thus far towards understanding the mechanism of ADE.

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DEVELOPING A MOUSE MODEL OF SECONDARY DENGUE VIRUS INFECTION

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Primary infections with dengue viruses (DENV) are typically asymptomatic or result in self-limited dengue fever (DF). Upon second infection with a different DENV serotype, however, patients are much more likely to develop life-threatening Dengue Hemorrhagic Fever (DHF) or Dengue Shock Syndrome (DSS). Increased disease severity in secondary DENV infections is believed to result at least in part from the phenomenon of antibody-dependent enhancement (ADE), in which anti-DENV antibodies are present at levels high enough to bind to the virus, but too low to efficiently neutralize it. Uptake of virus-antibody complexes by Fcγ receptor-bearing immune cells could then result in increased infection of these cells, which may lead to increased viremia and contribute to pathogenic immune dysfunction. Many aspects of the ADE phenomenon remain poorly understood, however, due to the lack of an appropriate small animal model. To explore the mechanism of secondary DENV infection *in vivo*, two approaches were used. In the first model, interferon α/β x γ receptor-deficient (AG129) mice were not infected or infected with the DENV1 Mochizuki strain at 5-6 weeks of age, then administered a secondary infection with DENV2 strain PL046 5 or 15 weeks later. At both time intervals, primary infection with DENV1 appeared to be protective in that reduced viral load was observed upon DENV2 infection of DENV1-immune mice compared to naïve mice. Other combinations of virus strains and longer intervals between sequential infections are currently being tested. In the second model, anti-DENV1, 3, or 4 immune serum was generated and tested *in vitro* for binding, neutralizing and enhancing activity against the homologous virus as well as DENV2. Antisera with enhancing activity *in vitro* will be passively transferred into AG129 mice prior to infection with DENV2. Viral load will be measured in various tissues to determine if the antisera enhanced infection. These approaches should shed light on the mechanism of ADE and the pathogenesis of secondary DENV infection *in vivo*.

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DENGUE VIRUS TARGETS MACROPHAGES AND DENDRITIC CELLS IN A MOUSE MODEL OF INFECTION

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Dengue fever is a mosquito-borne viral illness caused by one of four dengue viruses (DENV1-4), resulting in ~100 million infections each year in tropical and sub-tropical regions. A percentage of symptomatic infections proceed to a more severe, life-threatening form characterized by increased vascular permeability, designated dengue hemorrhagic fever/dengue shock syndrome. The molecular basis of DENV pathogenesis is not well understood, however, due in part to lack of a representative animal model in which to test hypotheses generated by clinical and epidemiological observations. The cellular tropism of DENV in humans has not been fully defined, although viral antigen has been detected in dendritic cells in skin biopsies and in monocytes from peripheral white blood cells (WBCs). We find that the initial cellular tropism of DENV in mice and humans is similar, even though the phenotypic endpoint of DENV infection in mice is usually encephalitis. 129/Pas mice lacking IFN-α/β and -γ receptors (AG129) were infected with DENV via a subcutaneous (sc) route to approximate a mosquito bite. Two DENV2 strains were used: a Taiwanese clinical isolate (PL046) as well as a mouse-passaged virus recently derived in our laboratory from PL046 (D2S10) that results in increased vascular permeability in mice. During the first week after sc infection with either DENV2 strain, virus is present primarily in the lymph nodes, spleen, bone

marrow, and circulating WBCs as measured by plaque assay. Of interest, the D2S10 strain displays an increased titer of virus in the bone marrow and WBCs at early timepoints post-infection. Flow cytometric analysis demonstrates that $CD11b^+$ macrophages and $CD11c^+$ dendritic cells are targeted by DENV2 *in vivo*. We have also detected infectious DENV and viral RNA by plaque assay and strand-specific RT-PCR, respectively, in both $CD11c^+$ and $F4/80^+$ spleen cells that were separated using antibody-labeled magnetic beads. In addition, bone marrow-derived macrophages from AG129 mice are susceptible to DENV2 infection *in vitro*, and increased titers of virus are recovered when sub-neutralizing levels of anti-DENV2 antibodies are present. Our data indicates that the initial cellular targets of DENV in mice and humans are the same -- primarily macrophages and dendritic cells, thereby allowing investigation of the tropism and pathogenesis of the virus at the cellular level in primary and secondary DENV infections in an *in vivo* mouse model.

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PHENOTYPING OF PERIPHERAL BLOOD MONONUCLEAR CELLS INFECTED BY DENGUE VIRUS IN PEDIATRIC CASES

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Although dengue virus (DENV) and DENV antigen have been detected in peripheral blood mononuclear cells (PBMC) *in vivo*, controversy remains regarding the major cell type infected and the frequency of infected cells. To identify which sub-populations of PBMCs are infected by DENV in humans with symptomatic dengue illness, flow cytometric analysis was performed on samples from pediatric cases of dengue in Nicaragua. A prospective study was conducted in the National Pediatric Reference Hospital in Managua from August 2005 to January 2006. Blood samples were obtained daily from suspected dengue cases recruited $\leq$ 4 days since onset of symptoms, and PBMCs were prepared using CPT tubes (ficoll/hypaque separation). Dengue virus antigens were identified using antibodies directed to the prM protein (monoclonal antibody 2H2). PBMCs were phenotyped using 2 cocktails of antibodies, one containing labeled monoclonal antibodies directed to CCR7, CD209, CD16, CD83, and CD86, and the other consisting of labeled antibodies targeted to CD8, CD4, CD32, CD14, and CD11c. Positive samples were then further analyzed by staining with CD20, as well as intracellular cytokine staining. The presence of DENV was also tested by RT-PCR and virus isolation in serum samples obtained from the patient at the time of PBMC collection. In preliminary results, PBMCs from 18/20 samples were positive for DEN antigen by 2H2 staining. All cells positive for DENV antigen expressed CD86 (the co-stimulatory molecule B7-2) and CD32 (FcγRII receptor). Increased CD209, CCR7, and CD16 (FcγRIII) expression appeared to correlate with higher levels of DENV antigen. These initial studies indicate that the sub-population of PBMCs infected by DENV is predominantly monocytes/dendritic cells, perhaps activated in the presence of immune complexes. In addition, DC-SIGN may not be the major DENV receptor of PBMCs *in vivo,* since the percentage of cells positive for DC-SIGN was much lower than the total percentage of infected cells. PBMCs from additional patients as well as serial daily samples are currently being evaluated using the same methodology.

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DIFFERENT SUBSETS OF PRIMARY HUMAN CELLS HAVE DIVERGENT SUSCEPTIBILITY TO DENGUE VIRUS (DV) INFECTION AND CAPACITY TO MEDIATE ANTIBODY-DEPENDENT ENHANCEMENT (ADE)

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ADE is postulated to be a major mechanism for causing dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). What specific cells mediate ADE, however, have been controversial. Many cell types have been implicated in ADE, including monocytes/macrophages, dendritic cells, Langerhans cells, endothelial cells, T cells, B cells and hepatocytes. The relative DV permissiveness among these cells and their intrinsic ability to mediate ADE are not yet known. To address these issues, we exposed freshly isolated human peripheral blood mononuclear cells to a virulent strain of DV2 (16681) in the presence or absence of pooled dengue-immune human sera (PHS), and assessed infection using novel monoclonal antibodies (MAb) against NS1 or E proteins by 8-10 color flow cytometric analysis. In parallel, we measured DV infection by conventional Western blot and reverse-transcriptase PCR techniques. In 10 healthy donors, we found that monocytes were the principal cells for DV infection without PHS (3.85$\pm$3.54% positive for anti-E MAb staining), and have the greatest capacity to mediate ADE (9.99$\pm$6.29%) in the presence of highly diluted PHS. In contrast, no T or B cells were infected with or without the addition of PHS (<0.02%). Interestingly, some cells lacking T, B or monocyte markers were also infected (0.36$\pm$0.15%) in the presence of highly diluted PHS. Furthermore, subsets of monocyte are not equally susceptible to DV infection, nor do they have the same capacity to mediate ADE. These results provide more insight into the pathogenesis of DHF and DSS.

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FORMULATION DEVELOPMENT OF A CHIMERIC MALARIA VACCINE CANDIDATE (PFCP2.9) WITH MONTANIDE ISA 720 FOR CLINICAL EVALUATION

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PfCP2.9 is a recombinant fusion protein consisting of AMA1 (domain III) and MSP1-19 that is undergoing development and evaluation as a vaccine candidate against *Plasmodium falciparum* malaria. Both AMA1 and MSP1 are located on the merozoite surface and are believed to play a role in the invasion of red blood cells. PfCP2.9 is secreted at high levels from GS115 *Pichia pastoris* with a yield of 1g/L at 30-L scale fermentation. More than 30% of the protein is recovered from a purification process involving phenyl hydrophobic interaction, ion-exchange, and gel filtration chromatography, with >98% purity. To date, the PfCP2.9 bulk remains stable at -70°C for at least one year. PfCP2.9 is undergoing Phase 1 clinical evaluation with Montanide ISA720. PfCP2.9 is emulsified with ISA 720 by homogenization to form a white water-in-oil emulsion with a mean particle size of approximately 1μM. The stability of the PfCP2.9/ISA720 formulation stored at 4°C is assayed by particle size distribution and SDS-PAGE of extracted antigen. The antigen remains unchanged for up to three months; thereafter, aggregation and degradation of PfCP2.9 is seen by SDS-PAGE of extracted antigen and this increases over time. After nine months, only 50-60% of the extracted antigen is full length. The addition of glycine to the PfCP2.9/ISA 720 formulation was shown to prevent modification of the antigen and to date, no modification of PfCP2.9 can be seen for up to nine months with storage at 4°C. We propose that the addition of glycine can stabilize the PfCP2.9/ISA 720 formulation and

should be included in all future clinical formulations with this antigen and adjuvant.

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A PHASE 1 DOUBLE-BLIND, RANDOMIZED, CONTROLLED STUDY OF AMA-1/MSP-1 RECOMBINANT MALARIA VACCINE (PFCP-2.9/MONTANIDE ISA 720): A BLOOD STAGE VACCINE FOR *PLASMODIUM FALCIPARUM* MALARIA

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A vaccine that can reduce both morbidity and mortality from ***Plasmodium falciparum*** infection is the goal of malaria vaccine development. Wanxing Bio-Pharmaceuticals reports on a Phase 1 double-blind randomized controlled trial of PfCP-2.9, a malaria vaccine candidate adjuvanted with Montanide ISA 720. PfCP-2.9 is a recombinant chimeric protein expressed from ***Pichia pastoris***, consisting of Apical Membrane Antigen-1 (domain III) and the 19 kDa portion of Merozoite Surface Protein-1 from the 3D7 and K1 ***P. falciparum*** lines, respectively. The primary objective of the trial at the Shanghai Changhai Hospital in China is to assess the safety and reactogenicity of PfCP-2.9/ISA 720 in healthy, adult volunteers. The secondary objective is to assess the vaccine's immunogenicity by evaluating and comparing antigen-specific antibody responses (anti-PfCP-2.9 ELISA) after each vaccination.

This trial, approved by three ethics committees, is being conducted in collaboration with the PATH Malaria Vaccine Initiative and the World Health Organization. Seventy volunteers who met the protocol-defined inclusion and exclusion criteria were randomly assigned to one of three dose cohorts (5 µg, 20 µg and 50 µg). Volunteers in the 5 and 20 µg cohorts were randomized a second time to one of two vaccination schedules (A: 0, 60, 180 days or B: 0, 90, 180 days). Volunteers in the 50 µg cohort were vaccinated according to schedule B. Within each of the five groups, 14 volunteers were randomized to receive either vaccine or control (10 volunteers received vaccine and four volunteers received Montanide ISA720 adjuvant alone). For both schedules A and B, the volunteers had 20 follow-up visits during their 240 days to check local and systemic adverse events (AE). Blood samples were taken for hematological and biochemical tests to assess safety, and for immunological analyses (anti-PfCP-2.9 ELISA, growth inhibition assay and immunofluorescence assay).

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PHASE 1 SAFETY AND IMMUNOGENICITY TRIAL OF $MSP1_{42}$-FVO/ALHYDROGEL AND $MSP1_{42}$-3D7/ALHYDROGEL BLOOD-STAGE MALARIA VACCINES IN US ADULTS

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A Phase 1 clincial trial has been conducted in healthy US adults to compare the safety and immunogenicity of 2 recombinant protein vaccines based on *Plasmodium falciparum* $MSP1_{42}$-FVO and $MSP1_{42}$-3D7, the 2 dimorphic forms of the blood-stage antigen. Volunteers were given 3 vaccinations of either 5, 20 or 80 µg protein adsorbed to Alhydrogel at 0, 1 and 6 months. All vaccinations were well tolerated. Antibody levels to homologous and heterologous $MSP1_{42}$, $MSP1_{19}$ and $MSP1_{33}$ were measured by ELISA pre-vaccination, collected 2 weeks after each vaccination and during the 6 month follow-up period. In a proportion of volunteers, elevated $MSP1_{42}$ specific antibody levels were present 2 weeks following the second vaccination and were boosted by subsequent vaccination. Following the third vaccination, $MSP1_{42}$ antibody responses varied from undetectable to a titer of over 3,000 (reciprocal dilution at an OD of 1). The elevated $MSP1_{42}$ specific antibody levels diminished with time prior to the six month boost but were still detectable 6 months following the final vaccination. No correlation between $MSP1_{42}$ antibody levels and vaccine dose was found. $MSP1_{19}$ specific antibodies were detected and correlated with $MSP1_{42}$ antibodies. Little $MSP1_{42}$ strain specificity in the anti-$MSP1_{42}$ and anti-$MSP1_{19}$ responses was detected by ELISA and immunofluorescence of infected red blood cells. This is in contrast to the $MSP1_{42}$ specific T cell responses described in a companion paper. Antibodies to the homologous $MSP1_{33}$ protein were observed following three vaccinations in some volunteers. No parasite growth inhibition was detected in an *in vitro* assay using purified antibody from the volunteers. The results of this clinical trial were not predicted by preclinical animal studies that showed $MSP1_{42}$-FVO was more immunogenic than $MSP1_{42}$-3D7 and a part of the antibody responses were strain specific. Due to the modest immune responses of $MSP1_{42}$/Alhydrogel vaccines, further development is underway to evaluate potentiation of the immune response by the addition of CPG 7909.

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T CELL RESPONSES IN VOLUNTEERS VACCINATED WITH BLOOD-STAGE MALARIAL ANTIGENS MSP-1 AND AMA-1

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The malaria merozoite proteins AMA-1 and MSP1 have been investigated as potential candidates for a malaria vaccine because of their pivotal role in the invasion of *Plasmodium falciparum* parasites into red blood cells. Since our knowledge of T cell responses in humans to these antigens is limited, we evaluated the nature and specificity of T cell responses in healthy American volunteers vaccinated with two leading blood-stage vaccine candidate antigens - $MSP1_{42}$ and AMA1-C1. Both proteins were formulated on Alhydrogel in two different Phase I Clinical trials. Antigen-specific T cell responses were assessed by ELISPOT assays, intracellular staining for IL-5 and IFN- , and ELISA determinations for several cytokines secreted upon re-stimulation with specific vaccine antigen. In MSP1 vaccinated volunteers, specific T cell immune responses were modest for Th1 cytokines such as IFN- or IL-2; however, Th2 cytokines like IL-5 and IL-13 showed increases after the third vaccination. Similar findings were observed in the volunteers vaccinated with AMA1- C1. These results indicate that the responses to alum adjuvanted vaccines in humans predominantly followed a Th2-type pattern. Two groups of $MSP1_{42}$ vaccinated volunteers were evaluated for allele specificity after one group was vaccinated with the $MSP1_{42}$ FVO allele and another was vaccinated with $MSP1_{42}$ 3D7. Interestingly, when responses to homologous and heterologous antigen were compared in each group, IL-5 responses were significantly higher for the homologous antigen, both in supernatants, and in the number of IL-5 secreting cells. In contrast, antibodies from the same individuals showed nearly complete cross-reactivity. This suggests that the T cell responses are more allele-specific than the B cell responses. The same malarial antigens formulated in Alhydrogel with CPG 7909 were also tested in Phase 1 Clinical Trials, and the specific cellular responses were evaluated. Results will be presented on the implications for the choice of antigens and adjuvants for candidate malaria vaccines.

(ACMCIP Abstract)

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RANDOMIZED, CONTROLLED, PHASE 1 STUDY OF AMA1-C1/ ALHYDROGEL® VACCINE FOR *PLASMODIUM FALCIPARUM* MALARIA IN CHILDREN IN DONÉGUÉBOUGOU, MALI

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Apical membrane antigen-1 (AMA1) - one of the leading malaria vaccine candidates - is a surface protein expressed during the asexual blood stage of *Plasmodium falciparum* that has been implicated in parasite invasion of erythrocytes. AMA1-C1/Alhydrogel® consists of an equal mixture of recombinant AMA1 from the FVO and 3D7 clones of *P. falciparum* that is adsorbed onto Alhydrogel®. A Phase 1 study in semi-immune adults in Mali has shown that the vaccine was safe and immunogenic particularly in those who received the 80 µg dose. A Phase 1 study in healthy children aged 2-3 years was started in March 2006 in Donéguébougou, Mali. Thirty-six children were enrolled into one of the two-dose-groups (n=18/ group) and randomized 2:1 to receive either AMA1-C1/Alhydrogel® or *Haemophilus influenzae* type b Hiberix® vaccine. The 1st and 2nd dose-groups were vaccinated successively at 3-week intervals, and received 20 and 80 µg of AMA1-C1, respectively. Vaccinations were administered on days 0 and 28 and participants were examined on days 1, 2, 3, 7, and 14 after each immunization and then about every two months for 1 year. Of 36 volunteers enrolled, 33 received both vaccinations. As of study day 42 of the second group, there have been 16 local, systemic and laboratory abnormalities related to the vaccination. All have been mild. Seven participants had elevated alanine-aminotransferase (ALT) due to acute Hepatitis A infection; three of these children were hospitalized briefly. No vaccine-related serious or grade 3 adverse events have been observed. There was no increase in local, systemic or laboratory abnormities with respect to increasing dose of vaccine or increasing number of immunizations. The blinded data so far suggest that AMA1-C1/ Alhydrogel® is well tolerated in children. Based on these safety results, a Phase 2 study in children is being undertaken. Additional safety results up to Day 154 will be presented.

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HUMAN MALARIA-SPECIFIC IFN-Γ T CELL RESPONSES INDUCED BY VIRUS LIKE PARTICLES, COMPRISED OF HEPATITIS B VIRUS CORE ANTIGEN AND *P. FALCIPARUM* CIRCUMSPOROZOITE PROTEIN T AND B CELL EPITOPES (ICC-1132), ADJUVANTED WITH ALUM AS COMPARED TO ISA 720

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Virus like particles (VLP) can provide a promising malaria vaccine platform as shown by recent clinical trials of RTS,S, a VLP comprised of Hepatitis B surface antigen and truncated *Plasmodium falciparum* CS protein. RTS,S vaccine efficacy required a complex adjuvant formulation containing an oil-in-water emulsion, QS21 and monophosphoryl lipid A. We have investigated a VLP vaccine comprised of recombinant Hepatitis B core antigen containing minimal T and B cell epitopes of *P. falciparum* CS protein (ICC-1132). In previously reported Phase I trials, ICC-1132 elicited positive anti-sporozoite antibody and CS-specific T cells in the majority of vaccinees. A critical role of CS-specific IFN-g producing CD4+ T cells in resistance to *P. falciparum* sporozoites has been shown in recent studies in vaccinated and naturally-infected humans. We therefore examined the kinetics and fine specificity of human IFN-g producing CD4+ T cells induced by multiple doses of ICC-1132/alum as compared to responses obtained following a single dose of ICC-1132 formulated in the more potent water-in-oil adjuvant, Montanide ISA 720. The majority of volunteers primed and boosted with ICC-1132/alum developed CS-specific T cells detectable by cultured IFN-g ELISPOT and by measurement of IFN-g in supernatants using the Cytokine Bead Assay (BD Biosciences). A single dose of vaccine formulated in ISA 720 also elicited malaria-specific IFN-g producing T cells detectable by these assays. The majority of cells were specific for the T* universal T cell epitope, with minimal responses to the CS repeats. In both adjuvant formulations, a Th1/Th0 cytokine profile predominated, with IFN, IL-2 and TNFα, but minimal IL4/ 5, detectable in the majority of culture supernatants. These findings suggest that the ICC-1132 VLP vaccine formulated in readily available adjuvants can induce CD4+ T cells that can target the parasite at multiple stages by secreting helper factors for production of sporozoite neutralizing antibody and by IFN-g mediated inhibition of intracellular hepatic stages.

(ACMCIP Abstract)

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INVASION INHIBITION OF *P.VIVAX* BY ANTI-DUFFY BINDING PROTEIN ANTIBODIES

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Plasmodium vivax (Pv) is the second most prevalent malaria species infecting humans accounting for half of all malaria cases in Latin America and Asia, and 70 million cases annually. Numerous studies have shown that Pv merozoites interact with the Duffy blood group antigen in order to complete the red blood cell (RBC) invasion process. Additional studies have identified the parasite binding ligand, Pv Duffy Binding Protein (PvDBP) and amino acids that interact directly with the human Duffy blood group antigen. Interference with, or disruption of this ligand:receptor interaction may limit Pv invasion of target reticulocytes, making the PvDBP an attractive vaccine candidate. To examine the influence of antibodies on Pv viability we tested two antibody solutions specific for the PvDBP Sal I allele for their ability to inhibit Pv invasion in short-term *in vitro* cultures. Parasites for these cultures were obtained from Pv-infected individuals from the Mae Sot region of Thailand. Following depletion of leukocytes (CF11 cellulose column), parasite cultures were established in cultures in McCoys 5A + 25% Human AB serum using autologous RBC. Antibodies included a rabbit polyclonal antisera raised against recombinant PvDBP, and an antigen-specific, affinity purified, pooled antisera from 14 Pv-infected Papua New Guineans. Results from our studies found that addition of rabbit anti-PvDBP (1:100) to short-term Pv cultures reduced the number of invasion events by an average of 66% (p=0.07). Affinity-purified antisera from Pv-infected Papua New Guineans (100 ug/mL) reduced Pv invasion by 43% (p=<0.01). These results show for the first time that both rabbit and human antibodies directed against the PvDBP Sal I allele reduce RBC invasion efficiency by Pv.

(ACMCIP Abstract)

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HUMAN HOOKWORM VACCINE TRIAL: MODELING TRIAL EFFICACY AND HEALTH IMPACT

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To help develop a new tool for the control of human hookworm, the Human Hookworm Vaccine Initiative (HHVI) has identified and produced several vaccine candidates, the most promising being the Na-ASP-2. The first-in-man study has been completed in healthy US volunteers and a phase 2 trial in an endemic area of Brazil is currently in preparation. The vaccine is expected to be non-sterilizing, but still capable of slowing down the post-treatment re-infection process, after chemotherapy. Little is known however about the efficacy and waning time both at the individual and population level, and no reliable animal model for this infection exists. To help explore the possible trial results and potential global health impact, we are developing a mathematical framework to quantify the consequences of vaccination and to define the desired characteristics of an efficacious. As an initial step, we investigate putative ecological and immunogenetic factors underlying observed patterns in hookworm infection, through a tailored individual-based computer simulation. This allows for a stratified analysis of the population and for the exploration of different scenarios, dependent on biological determinants (worm fertility and death rate, human genetic predisposition and age, environmental factors) as well as on behavioral factors (individual habits and social mixing). As a function of level of coverage, efficacy and waning-time, we estimate the direct benefit provided by the vaccine. A special emphasis is put on the role played by children and adult sub-populations in the hookworm transmission dynamics and on the determinants and epidemiological consequences of spatial heterogeneity of infection. A sensitivity and sample-size dependency analysis provide focus for the design and the analysis of the vaccine trial. We find that both immunogenic and behavioral heterogeneities are likely to play crucial, although different, roles in the dynamics of hookworm infection and will both need to be taken into account when choosing size and (possibly) composition of the vaccine trial population sample. In addition, we identify the vaccine efficacy and waning required for the vaccine to have a substantive impact on mean worm relative to proving chemotherapy alone.

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PARASITE RISK FACTORS FOR UNDERWEIGHT AND WASTING IN PRESCHOOL-AGE CHILDREN IN BELEN, PERU USING THE NEW WHO INTERNATIONAL GROWTH STANDARDS

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Background: Recently WHO released a new set of international growth standards with which more accurate estimates of malnutrition could be calculated. This information is particularly valuable in areas of extreme poverty as it can ensure that risk factors for malnutrition, including parasite infections, are properly identified and appropriate interventions targeted. The objective of this study was to determine accurate estimates of malnutrition indicators and associated risk factors in a population of preschool-age children in Belen, Peru, a community of extreme poverty. A household survey was conducted in Belen from Oct 2005 to Jan 2006. Demographic information and various measurements (anthropometry, blood and stool samples) were collected from one child under five from each household. Anthropometric calculations were made using the WHO Anthro 2005 software. Wasting and underweight were defined as > -2 SD from the WHO reference population for height-for-weight and weight-for-height, respectively. Multivariable logistic regression was used to determine the independent risk factors for wasting and underweight. A total of 252 children with complete anthropometric measurements, and blood and stool samples were included in the analysis. Mean age was 27.5 months (±16.2). Forty-nine percent were female. The prevalence of wasting and underweight was 26.6% and 28.6%, respectively. Risk factors for underweight included mother's education level (secondary incomplete vs. secondary complete) (OR=3.04; 95%CI: 1.47, 6.29), moderate-high intensity of Trichuris infection (OR=4.78; 95%CI: 2.08, 11.02), and age of child (OR5-month=1.14; 95%CI: 1.04, 1.26). The above risk factors and one additional risk factor (hookworm infection (OR=6.41; 95%CI: 1.08, 38.5)) were also associated with wasting. The results indicate the presence of parasite risk factors for malnutrition in children under five in this community of extreme poverty. Cost-effective strategies for deworming and nutrition interventions that target mothers and preschool children are urgently required.

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RECENTLY IDENTIFIED *BACILLUS* SPECIES PRODUCER OF POTENT NEMATOCIDAL COMPOUNDS

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Nematode diseases including elephantiasis and oncherciasis still persist, and affect millions particularly in developing countries. Present drugs including Mebendazole and Diethylcarbamazine are somewhat effective. We continue to investigate the effects of the purified component of an extract obtained from *Bacillus mojavensis* strain 14135 nov. The fractionated protein was analyzed utilizing GC/MS. Varying concentration of the purified fraction was added to culture-plates containing 1st through 4th stage larvae of *Caenorhabditis elegans* (*C. elegans)*. This study examines the effects of these various concentrations on different larvae of *C. elegans*. 1st to 4th stage larvae of *C. elegans* were cultured in Lauria broth in 24 well tissue-culture plates. The medium was supplemented with 5mg/ml cholesterol and OP50 strain *E. coli* previously cultured in 2xYeast Tryptone broth. We subsequently treated test wells with varying concentration of the fraction from.05µg/ml-10.0µg/ml. The fractions contained a number of recently identified lactones including Pyrrolo [1, 2-a] piperazine-3, 6-dione and Pyrollo [1, 2-a] pyrazine-1, 4-dione. We checked for the lack of motility in the different larval stage over a 72-hour period, and examined worms for structural damage. We recorded the absence or presence of motility which begun at approx. 15 minutes post-treatment. Muscular activity appeared to be totally absent after the 72 hours. Later larval stages appeared to be affected more readily. However, all larval stages subsequently succumbed to the compounds within the extract. Conclusions: It appears that the compound(s) Pyrrolo [1, 2-a] piperazine-3, 6-dione and Pyrrolo [1, 2-a] pyrazine-1, 4-dione which were identified by GC/MS fractionation are exceedingly toxic to all larval stages. Further studies to understand the activity of the identified compound(s) will be conducted. However, it appears that these compound(s) could be of tremendous importance in the future regarding the fight against several nematode infections and other parasitic organisms.

(ACMCIP Abstract)

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THE INFLUENCE OF HELMINTHS ON IMMUNE RESPONSES TO HIV

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Differences in the AIDS epidemic have been documented to exist between developing countries and the Western world. Several factors responsible for this variation have been suggested. Background immune activation by infectious agents common among poor communities of developing countries has been widely implicated. Helminths have been shown to play a significant role in inducing chronic immune activation in these populations. Increased susceptibility to HIV infection and faster progression to AIDS are postulated to result from pre existent immune dysregulation under these conditions. The aim of this study was to define the immune profile of HIV-1 seropositive and seronegative individuals with or without helminth co-infection in a resource-poor setting. The hypothesis testes was that helminths alter immune responses to HIV infection by a shift from Th1 to Th0/Th2 phenotype and enhance susceptibility to HIV/AIDS. Baseline and follow up blood and fecal samples obtained from 128 HIV-1 seropositive and 45 HIV seronegative individuals were collected at intervals after a 15 month period of deworming. Evidence for helminth exposure was measured by fecal egg excretion and elevated Ascaris specific IgE. Lymphocyte phenotypes and viral loads were compared between groups infected or uninfected with helminths and HIV-1.Currnetly, activation status, lymphocyte proliferative responses and, cytokine production are investigated between subgroups. Fifty-three of 128 HIV-1 positive and 18 of 45 negative subjects had evidence of worm infection. Lower median CD4 and higher CD8 counts were obtained from HIV-1 positives compared to negatives. In the former, higher median viral loads were associated with helminth infection. However, higher CD4 counts were found in helminth-infected groups with or without HIV. In conclusion, these preliminary results are equivocal thus further analysis of the complete immune profile among the groups will elucidate whether helminth exposure alters the response to HIV infection.

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CYTOKINE RESPONSES TO STRONGYLOIDES STERCORALIS INFECTIVE-STAGE LARVAL ANTIGEN IN STRONGYLOIDIASIS PATIENTS WITH HTLV-1 CO-INFECTION

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The presentation of human strongyloidiasis varies between host populations from a chronic, but limited infection in normal hosts to hyperinfection in patients treated with corticosteroids or with HTLV-1 infection. In murine models, granulocytes (especially eosinophils) and antibody play key roles in controlling infection, but there are few data in human subjects. The objective of this study was to evaluate *Strongyloides* larval antigen specific cytokine responses in strongyloidiasis patients with differing underlying diseases. PBMCs were isolated from 8 newly diagnosed strongyloidiasis patients in Lima, Peru, 3 of them with HTLV-1 co-infection, and 2 uninfected controls. PBMCs were cultured in complete media in the presence or absence of crude *Strongyloides* larval antigen. Supernatants were collected and stored at -80°C until analysis by multiplex bead immunoassay. We observed spontaneous production of the Th1 type cytokines [IFN- (414.5 ± 36 pg/ml) and CXCL-10 (5031 ± 3295 pg/ml)] and the regulatory cytokine IL-10 (43.5 ± 29 pg/ml) in unstimulated PBMCs from strongyloidiasis/HTLV-1 co-infected individuals. By contrast, we noted spontaneous production of IL-17 in patients with strongyloidiasis only (27.2 ± 15.7 pg/ml). With antigen stimulation, patients with strongyloidiasis-only but not HTLV-1 co-infection produced IL-5 (566.7 ± 269 pg/ml for those with just strongyloidiasis versus 69.3 ± 64 pg/ml in those co-infected with HTLV-1) IL-13 antigen-specific response was preserved in both, strongyloidiasis and strongyloidiasis/HTLV-1 co-infected patients (237.6 ± 98.73 vs 255 ± 145 pg/ml). In conclusion, we were able to confirm spontaneous Th1 and regulatory cytokine production as reported before in HTLV-1 infected patients. Spontaneous production of IL-17 (involved in neutrophil production and recruitment) and antigen-driven production of IL-5 (involved in eosinophil production) further support the key role of granulocytes in control of strongyloidiasis.

(ACMCIP Abstract)

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MYELOPEROXIDASE IS REQUIRED FOR PROTECTIVE ADAPTIVE IMMUNITY TO *STRONGYLOIDES STERCORALIS* IN MICE

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Neutrophil recruitment is required for protective immunity against *Strongyloides stercoralis* infective stage larvae (L3) during both the innate and adaptive immune responses. Additionally, neutrophils are capable of killing L3 *in vivo*, also during both the innate and adaptive immune responses. To determine what neutrophil products are capable of killing L3, the neutrophil granule component myeloperoxidase (MPO) was tested as a representative oxygen-dependent antimicrobial enzyme while neutrophil elastase (NE) was tested as an oxygen-independent antimicrobial enzyme. MPO and NE knock-out (KO) mice were infected with *S. stercoralis* L3, and the ability of the mice to mount effective immune responses was monitored. NE KO and wild-type C57BL/6 mice had similar ability to kill L3 during both the innate and adaptive immune responses. MPO KO mice also exhibited no difference in larval killing versus wild type mice during the innate immune response. However, immunized MPO KO mice had a statistically significant reduction in the number of L3 killed versus wild type controls, despite normal recruitment of cells. These data suggest that neutrophil elastase is not required for the innate or adaptive immune response to *S. stercoralis*, while MPO is required for the killing of L3 by neutrophils in the adaptive response, but not during innate immunity.

(ACMCIP Abstract)

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POOR SANITATION AND HELMINTH INFECTION PROTECT AGAINST SKIN SENSITIZATION IN VIETNAMESE CHILDREN: A CROSS-SECTIONAL STUDY

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Allergic disease is uncommon in developing countries, especially in rural areas. A protective effect of geohelminth infection, among other environmental factors, has been implicated as a potential explanation. The objective of this study was to determine whether current helminth infection is associated with a reduced prevalence of allergen skin test sensitization in a South-East Asian population of children with a

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high prevalence of hookworm infection. All primary and secondary schoolchildren from four neighbouring communes in a rural district of central Vietnam were invited to take part in a cross-sectional survey. Allergen skin sensitization to housedust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), and American cockroach (*Blatella americana*) were measured and stool samples for qualitative and quantitative geohelminth estimation collected. 1,601 children age 6-18 participated. Sensitization to dustmites was present in 14.4%, and to cockroach in 27.6% of children. In univariate analysis, sensitization to either allergen source was less frequent in children with hookworm or *Ascaris* infection, and increased in those with better santitation, including flush toilets and piped drinking water. In a mutually adjusted model, the risk of sensitization to dustmites was reduced in those with higher hookworm burden (adjusted OR for 350+ versus no eggs per gram=0.61, 95% CI 0.39-0.96), and with *Ascaris* infection (adj OR=0.28, 0.10-0.78), and increased in those using flush toilets (adj OR for flush toilet versus none/bush/pit=2.51, 1.00-6.28). In contrast, sensitization to cockroach was not independently related to geohelminth infection but was increased in those regularly drinking piped or well water rather than from a stream (adj OR=1.33, 1.02-1.75). In conclusion, geohelminth infection, sanitation and water supply influence the risk of allergic sensitization in Vietnamese children. These findings are consistent with a protective effect against allergy by geohelminth or other gastrointestinal infection.

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