those in people with no history of dengue. Our results suggest that the level of antibodies can be used as a proxy for mosquito bite exposure and a measure of dengue fever risk.

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EVALUATION OF PREDICTIVE MAPS FOR *AEDES AEGYPTI* LARVAL HABITATS IN TWO URBAN AREAS OF COSTA RICA

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The abundance of *Aedes aegypti* can be associated with urban structure and tree cover, which conceals and protects containers. The purpose of this study was to create and evaluate predictive maps for *Ae. aegypti* larval habitats in Puntarenas and Carpio, two very different urban environments in Costa Rica. Linear regression models for number of mosquito larval habitats had been developed for Puntarenas, and they showed a significant association with tree cover when corrected by the number of locations evaluated (R^2 = 0.650, p<0.001). Land cover maps were created from Quickbird satellite imagery of both sites. Data was extracted from 50 by 50 m cells, and parameters from the model were used to create predictive maps by determining the expected number of *Ae. aegypti* positive larval habitats in all cells that cover the urban areas. To evaluate maps, cells were randomly selected, and entomological evaluations were performed. Four categories were created for the number of larval habitats per cell: low (0-1), medium (2-3), high (4-5), and very high (6 or more). For both sites, the expected number of wet containers in sample cells fell within the 95% confidence interval of predicted values. In Puntarenas, 382 wet containers were identified, container index was 22.5% and Breteau index 43.7. Expected and observed categories of *Ae. aegypti* larval habitats per cell in Greater Puntarenas were significantly correlated (p=0,037). Only 32.5% of cells harbored the exact number of expected habitats, 74% contained the expected number +/- 2 habitats, and only 16% underestimated total larval habitats. In Carpio, 693 wet containers were identified, container index was 11.4% and Breteau Index 24.7. Expected and observed categories of *Ae. aegypti* positive habitats per cell were not significantly correlated in Carpio. Only 50% of cells contained the expected number +/- 2 habitats, and 29% underestimated the total observed. The most frequent *Ae. aegypti* larval habitats in Puntarenas included outdoor containers and miscellaneous objects, while larval habitats in Carpio were commonly human-filled, such as drums and buckets. These maps and models may be considered adequate for areas like Puntarenas, whereas they do not seem to apply for Carpio. Tree cover may provide useful information in sites where *Ae. aegypti* larval habitats include mostly outdoor rain-filled containers, as opposed to sites where containers are greatly affected by the need for water storage.

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CLIMATIC VARIABILITY AND LANDSCAPE HETEROGENEITY IMPACT URBAN MOSQUITO DIVERSITY AND VECTOR ABUNDANCE AND INFECTION

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Urban habitat heterogeneity can modify patterns of interactions across species and lead to spatially fine grained differences in β-diversity patterns and their associated ecosystem services. Here, we study the impacts of landscape heterogeneity and climatic variability on: (i) the richness and diversity patterns of mosquitoes (Diptera: Culicidae) and (ii) the abundance and West Nile virus infection rate of the house mosquito, Culex pipiens, in Chicago, USA. We conducted a four year long study (2005-2008) in 8 sites that captured a gradient of urban heterogeneities. We found a total of 19 mosquito species, a representative sample of mosquito species richness in the area, according to both model estimation (Chao2 ± S.E. = 20.50 ± 2.29) and faunal records for Chicago. We found that heterogeneity in the landscape was the best predictor of both mosquito species richness and diversity, with the most heterogeneous landscapes harboring the largest number of species. In general there were no changes in species richness over the years that could be associated with weather patterns and climatic variability (WPCV). In contrast, changes in diversity evenness showed signatures of WPCV. Our results also showed that WPCV had major impacts on house mosquito abundance and West Nile virus mosquito infection rate (MIR) patterns. Although MIR was independent of mosquito diversity, it was associated with overall mosquito abundance, which had a convex association with species richness (i.e., abundance increases to a point after which it decreases as function of species richness). Finally, our results highlight the importance of considering dominant vector species as part of a community of vectors, whose biodiversity patterns can directly or indirectly impact the risk of infectious disease transmission.

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NON-LINEAR IMPACTS OF CLIMATIC VARIABILITY ON *AEDES AEGYPTI* POPULATION REGULATION

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Aedes aegypti is one of the most common urban tropical mosquito species and an important vector of dengue, chikungunya, and yellow fever viruses. It is also an organism with a complex life history where larval stages are aquatic and adults are terrestrial. This ontogenetic niche shift could shape the density dependent regulation of this and other mosquito species because events that occur during the larval stages impact adult densities. Here, we present results from simple density-dependence mathematical models fitted using maximum likelihood methods to weekly time series data from Puerto Rico and Thailand. Density dependent regulation was strong in both populations. Analysis of climatic forcing indicated that populations were more sensitive to climatic variables with low kurtosis (i.e., climatic factors highly variable around the median) rainfall in Puerto Rico and temperature in Thailand. Changes in environmental variability appear to drive sharp changes in the abundance of mosquitoes. The identification of exogenous factors forcing the sharp changes in disease vector populations using their statistical properties, such as kurtosis, could be useful to assess the impacts of changing climate patterns on the transmission of vector-borne diseases.

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THE ROLE OF SWINE IN THE ECOLOGY OF JAPANESE ENCEPHALITIS VIRUS TRANSMISSION OF SOUTHERN VIETNAM

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Japanese encephalitis virus (JEV) is a mosquito-borne flavivirus disease of major public health importance and is endemic to both north and south Vietnam. Swine populations play a role in JEV transmission as both a reservoir and amplifying host. In general, infected adult pigs support transient but high titer viremias and remain asymptomatic. In contrast, naïve piglets exhibit fever and experience weight loss, and gilts or sows who become infected from 40-80 days of gestation often abort or give birth to stillborn mummified fetuses. In some JEV-endemic countries, swine vaccination is performed within commercial livestock sector to prevent losses in reproductive performance of breeding herds. Here we review previous unpublished studies of JEV seroprevalence

within Vietnamese swine from the 1970s and 1990s, as well as novel data generated in our lab on seroprevalence of swine from southern Vietnam from 2006-2010. We discuss the potential utility of measuring JEV seroconversion in sentinel pigs for monitoring virus circulation in nature, and geographic targeting of childhood vaccination strategies. Furthermore, the swine seroprevalence data are used as input parameters for models of disease transmission dynamics, to examine the relationships between hyperendemicity, herd immunity, and the emergence of symptomatic disease.

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CLUSTER RANDOMIZED CONTROLLED TRIAL TO DETERMINE THE ADDITIONAL BENEFITS OF TOPICAL REPELLENTS TO LONG LASTING INSECTICIDE NETS (LLINS) ON MALARIA INCIDENCE

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Long lasting insecticide nets (LLINs) is the most effective tool against malaria vectors. Extensive LLINs coverage may give communal protection to non-users, by reducing vector populations or diverting them to other hosts. On the other hand LLINs may force mosquitoes to feed earlier in the evening and thus reducing the efficacy of LLINs or even divert mosquitoes to non-users, putting them at a higher risk of malaria. Several studies have shown significant malaria reduction in groups that use LLINs plus repellents versus LLINs only in areas where early evening malarial vector exposure occurs. This study tested whether repellents, used in combination with LLINs, can provide additional protection from malaria in an area of high LLINs use and early evening exposure in rural Tanzania. Data on the efficacy of deet (N, N-Di ethyl-3-methyl-benzamide) repellent against mosquito bites was collected in semi-field trials and field trials carried out in rural Tanzania. A 3 × 3 Latin square was used in both these trials and data was analyzed using Generalized estimating equations (GEE). Data on clinical outcomes was collected using a blinded cluster-randomized controlled trial measuring malaria incidence by passive case-detection in the intervention arm, (LLINs plus15% topical deet repellent) and control arm (LLINs plus placebo lotion). The trial was designed to have 80% power, to detect a 24% treatment effect at 95% confidence interval and a correlation co-efficient of 0.25. Compliance was measured using questionnaires. Malaria was tested using rapid diagnostic tests at a local clinic. Clinical malaria incidence rates, measured in person years were the outcome. Socio-economic parameters were measured by PCA. The results of the efficacy trials show that 15% deet repellent is 90% protective against all mosquito species under field conditions, and 70% protective against high biting density of *An. gambiae* in semi-field conditions over a period of 4 hours. Clinical data demonstrated a reduction of malaria in the intervention arm, though this difference was not significant.

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ENTOMOLOGICAL MONITORING AND EVALUATION OF INDOOR RESIDUAL SPRAYING IN UGANDA

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Malaria prevalence is high in Northern Uganda particularly in children under five years of age where *Anopheles gambiae s.s.* and *An. funestus* are the main vectors. To bring malaria under control, a large scale indoor residual spraying (IRS) program with Bendiocarb 80% wettable powder at 0.4 g/m2 was conducted from June 2010 to March 2011in six highly malaria endemic districts. During this period, two consecutive spray cycles were conducted in each district, with 5-6 months intervals. Pyrethrum spray catches (PSCs) were performed two to three weeks prior to and after IRS in 18 sentinel sites (12 houses /site) to assess the impact of IRS on vector indoor resting densities. World Health Organization wall cone bioassays were also conducted to measure the quality of spraying immediately after IRS, and at monthly intervals thereafter to track the residual efficacy of the insecticide on sprayed surfaces following the spraying. The PSC result shows that spraying Bendiocarb significantly reduced vector indoor resting densities in all households in both spray rounds. In round one, pre and post IRS-indoor resting densities were 12.33 and 0.097per house respectively ($p<0.001$). In round two, pre-IRS indoor densities were still low, 0.305 per house after 4-5 months following round one and reduced to 0.0972 per house thereafter in round two post IRS($p<0.001$), suggesting that the subsequent spraying of Bendiocarb at 5-6 month intervals further reduced the vector densities. The bioassay tests conducted soon after IRS showed 100% knock-down (KD) rates and 24-hour mortality in all tested houses, indicating high quality IRS. The monthly bioassays revealed that the insecticidal effect of Bendiocarb remained at 100% for three month period and dropped to 80% (24 hour mortality) in the fourth month after IRS. This study suggests that entomological monitoring to measure the impact of indoor residual spraying on vector population is useful and routine PSC as well as cone bioassays could also be used as an operational tool for programmatic decisions in IRS programs.

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EVALUATION OF ZEROVECTOR® DURABLE LINING (DL) - IMPACT ON *AEDES AEGYPTI* AND *ANOPHELES STEPHENSI* UNDER VARYING DL COVERAGE

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Recently, a Durable Activated Residual Textile (DART) product has been developed as an alternative to the traditional indoor residual spray (IRS) strategy. The function of this durable-lining (DL) material is to transfer toxic doses of deltamethrin to vectors resting on the surface of the material placed along the interior walls of homes thereby reducing overall populations and biting pressures to human hosts. Our previous studies focused on the resting, escape and mortality behavior of non-bloodfed (i.e. host-seeking) *Ae. aegypti* test cohorts using varying coverage levels of DL material to describe the negative effects of "safe-sites" (areas of the wall where DL is not present) on overall efficacy. Here we present results on continued studies describing the resting preference, knock down (KD) and 24 h mortality responses of bloodfed *Ae. aegypti* and unfed *Anopheles* stephensi females exposed to varying surface area coverage of DL (100, 75, 50 and 25%) under laboratory conditions. Studies evaluating the DL color preference of *Ae. aegypti* against blue and green DL were also performed. For all assays in which treated DL was applied, there was less resting response overall - even on safe sites (i.e., metal surfaces) within the assay device- and significant increases in the proportion of test cohorts flying and exhibiting KD compared to matched controls. This indicates an agitation response from the chemical active that was true even at a 25% coverage ratio. Mortality rates at 24h post-exposure from resting preference studies and KD rates from escape response assays indicate the DL product delivered a toxic dose of insecticide to both bloodfed *Ae. aegypti* and unfed *An. stephensi* assay populations at all surface coverage ratios. Overall, there was no significant DL color preference (either green or blue) observed under these test conditions. Combined, these results suggest minimal negative effects of color or safe sites to the overall efficacy goals of the DL product for both a dengue and malaria vectors. Similar studies will be repeated against bloodfed anopheline test cohorts, with field validation of laboratory findings currently in preparation.

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STABLE ISOTOPE ANALYSIS OF *AEDES AEGYPTI* AND *CULEX QUINQUEFASCIATUS* REVEALS LARVAL HABITAT SOURCES OF ADULT MOSQUITOES IN PUERTO RICO

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Larval habitats of the dengue virus vector, *Aedes aegypti*, are diverse and include ones above ground (such as water-filled pails, pails with water and plants, and discarded tires) and below ground (such as septic tanks). Quantifying which larval habitats contribute to the bulk of the adult vector population has proven difficult but is important to understand the basis for mosquito production and resultant virus transmission, and to design habitat-specific vector control programs. Stable isotope analysis provides a tool for determining the food resource base of mosquito larvae by evaluating habitat-specific 13C:12C and 15N:14N ratios. These habitat-specific ratios are retained in tissue of emergent adults; therefore, individual adults can be assigned to original larval habitats. In this study, we analyzed carbon and nitrogen stable isotope ratios of adult *Ae. aegypti* and *Culex quinquefasciatus* that had emerged from a diverse array of habitats in dengue-epidemic prone communities of southern Puerto Rico. Multiple logistic regression revealed habitat specific signatures and specifically separated below-ground (septic tank) and above-ground habitats for male and female adults of both species. Further, discarded tires were separable from water-filled pails and from pails with plants, all above-ground sources. Carbon ratios were particularly predictive as adults from septic tanks were enriched with 13C compared to adults from above-ground sources. Nitrogen isotope ratios were less predictive except that adults that had emerged from discarded tires had a higher 15N enrichment compared to other habitats. Carbon:nitrogen ratio in *Ae. aegypti* was higher than in *Cx. quinquefasciatus*, probably because the former species better withstands nitrogen limiting conditions. Overall, the carbon and nitrogen stable isotope dynamics likely reflect different nutrient inputs, microbial metabolic processes, and decomposition pathways operating in these different larval environments.

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DYNAMIC COMPONENTS OF THE MATING SYSTEM OF THE DENGUE VECTOR *AEDES AEGYPTI*

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Dengue is a major cause of hospitalization and death among children in Southeast Asia. Dengue virus is transmitted through the bite of infected *Aedes aegypti* females. There are approximately 50 million people infected by dengue annually (WHO 2010). In order to control this disease, we need to control mosquito populations. Understanding mosquito mating behavior and frequency, including male preference for females is an important key affecting mosquito population structure and genetic control efforts. We investigated the frequency of multiple mating and more specifically, the frequency of sperm transfer and female utilization of sperm from more than one mating. In this study, we report our results of female sperm usage patterns using a combination of approaches including PCR-based detection of sperm genotypes and screening of female reproductive output. In addition, little information is known about the preferences of free-ranging wild male *Ae. aegypti* for female body size. Mosquito mating patterns are a fundamental component of sexual selection and may have significant influences on the genetics and demographics of mosquito populations.

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COMPARATIVE EVALUATION OF ALTERNATIVE MOSQUITO SAMPLING METHODS IN LOWLAND SOUTHEAST, ZAMBIA

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Sampling malaria vectors is paramount for measuring malaria transmission. Human landing catch (HLC) has been the gold standard over the years but due to potential infective bites, attempts to develop alternative mosquito sampling methods have been made in malaria endemic countries. Centre for Disease Control and prevention miniature light trap (CDC-LT), Ifakara Tent Trap design model C (ITT-C), HLC outdoor, standardized resting boxes (RBO) and exit window traps (EWT) were evaluated against the standard HLC indoors in south east of lower Zambia. A 3 X 3 Latin square design was used with a series of ten rounds. Generalized Linear Model was applied to estimate mean catches to determine their efficiency and binary logistics to determine proportionality of abdominal status per sampling method. CDC-LT was 1.459 and 1.134 more sensitive than human landing catches indoor for sampling *Anopheles gambiae sensu lato* (s.l) (Giles) and *An. funestus* (Giles) species respectively. HLC outdoor, ITT-C had relative sensitivity (RS) of 0.594 and 0.040 for sampling *An. gambiae s.l* and 0.929 and 0.608 for *An. funestus* respectively. ITT-C collected more *An. funestus* than other mosquito species. Resting boxes and the exit window traps were the least efficient sampling methods. Proportionally 28.6% and 14.1% of blood fed *An. gambiae s.l* and *An. funestus* were collected respectively in ITT-C a tool supposedly to be exposure free. CDC-LT was the most efficient sampling method in the study site but routine monitoring of programmes may pose challenges ranging from availability of batteries and skilled manpower.

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BIOLOGICALLY MEANINGFUL COVERAGE INDICATORS FOR MALARIA VECTOR CONTROL INTERVENTIONS

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Indoor residual spraying (IRS) and insecticide-treated nets (ITNs) have been shown to dramatically reduce malaria transmission but cannot completely eliminate it from most settings in Africa. Although, mosquito species which predominantly feed on animals transmit malaria less efficiently, they are primary malaria vectors in many tropical countries and can dominate residual transmission in Africa where ITNs or IRS are widely used. While ITNs are known to confer personal protection against any mosquitoes attempting to bite while they are in use, it remains unclear whether they confer community-level protection for predominantly zoophagic vectors. Here we use a process-explicit malaria transmission model to assess the likely extent and mechanism of community-level impact of ITNs upon human malaria exposure to zoophagic vectors. Consistent with field observations in Africa, ITNs are most effective against anthropophagic vectors because the fraction of available blood that covered people represent is very high so that survival per feeding cycle is reduced, the length of feeding cycle is extended, and the emergence rate for adult mosquitoes is reduced. ITNs are less effective against zoophagic vectors because animals are available as alternative blood sources so negligible impact upon survival, feeding cycle length or reproduction rates occurs. Nevertheless, ITNs can deliver appreciable communal protection against transmission by zoophagic vectors so long as exposure predominantly occurs indoors. This is because the very small proportion of a zoophagic

vector population that get killed or diverted by ITN are the same proportion that actually transmit malaria so reducing an already low proportion of bloodmeals taken from humans has a substantial impact upon overall transmission. We also examine what coverage and protection really mean and how they determine the impact on real-world public health programmes. Further reduction of malaria transmission will require new methods for measuring and targeting blood resources other than indoor-resting humans, which mosquitoes depend upon for survival.

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TARGET PRODUCT PROFILE CHOICES FOR INTRA-DOMICILIARY MALARIA VECTOR CONTROL PESTICIDE PRODUCTS: REPEL OR KILL?

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The most common pesticide products for controlling malaria-transmitting mosquitoes combine two distinct modes of action: 1) conventional insecticidal activity which kills mosquitoes exposed to the pesticide and 2) deterrence of mosquitoes away from protected humans. While deterrence enhances personal or household protection of long-lasting insecticidal nets and indoor residual sprays, it may also attenuate or even reverse communal protection if it diverts mosquitoes to non-users rather than killing them outright. Here we describe a process-explicit model of malaria transmission which captures the sequential interaction between deterrent and toxic actions of vector control pesticides and accounts for the distinctive impacts of toxic activities which kill mosquitoes before or after they have fed upon the occupant of a covered house or sleeping space. Increasing deterrency of intradomiciary measures such as indoor residual spraying (IRS) and insecticide-treated nets (ITNs) increases personal protection but consistently reduces communal protection because deterrent sub-lethal exposure inevitably reduces the proportion subsequently exposed to higher lethal doses. If the high coverage targets of the World Health Organization are achieved, purely toxic products with no deterrence are predicted to generally provide superior protection to non-users and even users, especially where vectors feed exclusively on humans and a substantial amount of transmission occurs outdoors. Remarkably, this is even the case if that product confers no personal protection and only kills mosquitoes after they have fed. Products with purely mosquito-toxic profiles may therefore be preferable for ITN or IRS programmes with universal coverage targets, rather than those with equivalent toxicity but which also have higher deterrence. However, if purely mosquito-toxic products confer little personal protection, then they will require aggressive "catch up" campaigns, with behaviour change communication strategies that emphasize the communal nature of protection, to achieve high coverage rapidly.

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TUBERCULOSIS IN NINGXIA HUI AUTONOMOUS REGION, THE PEOPLE'S REPUBLIC OF CHINA

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Tuberculosis (TB) is a major cause of morbidity and mortality throughout the world. Practical control relies on rapid and effective diagnosis and treatment of active cases in order to protect vulnerable subjects and decrease transmission. China is the second most endemic country for TB, after India. Ningxia Hui Autonomous Region (NHAR), located in the north-west, is one of the poorest areas in China and national surveys have revealed a very high prevalence of human TB. A retrospective TB study we undertook of TB clinical records from NHAR further disclosed the serious nature of the infection there. The numbers of advanced TB cases were highest in two counties - Xiji and Guyuan with prevalences of 16.7% and 18.5%, respectively. More than 85% of active cases in Xiji County undertook treatment for over 12 months, although the remainder of the prolonged treatment cases appeared to correlate with neither disease stage nor disease incidence. Haiyuan and Tongxin counties had the highest incidence with the lowest population densities and the largest areas suggesting that they might account for the majority of TB transmission. On average between 10.9%-15.3% of NHAR patients were not diagnosed for 6 months in 2005-2009. This is of great public health concern and may account for increased TB transmission in these areas. The distribution of sputum-positive cases showed the lack of correlation with disease stage or incidence, which again suggests that there may be important epidemiological and socio-economic factors at play. From these data, we can conclude that there is a lack of community TB awareness and, in part, poor awareness and/or training in local health facilities which directly increases community exposure and fuels TB transmission. The data also showed a significant failure of DOTS which may reflect poor compliance. This again unavoidably promotes TB spread and will undoubtedly escalate the TB disease burden in future. Given the increase in population migration, the threat of TB is critical not only for NHAR and other parts of China but globally.

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EVIDENCE OF CROSS-REACTIVITY AGAINST AVIAN H5N1 AND PANDEMIC H1N1 2009 INFLUENZA VIRUSES FOLLOWING VACCINATION WITH A PRIME-BOOST REGIMEN OF SEASONAL INFLUENZA VACCINES

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Recent studies have demonstrated that inactivated seasonal influenza vaccines may elicit production of heterosubtypic antibodies, which can neutralize avian influenza H5N1 virus in a small proportion of subjects. We hypothesized that administration of a prime boost regimen of live and inactivated seasonal influenza vaccines would enhance the production of heterosubtypic antibodies and provide evidence of cross-protection against a range of influenza viruses. In a randomized open-label pilot feasibility study, 26 healthy adult volunteers were randomized to receive 1 of 4 vaccine regimens containing two doses of 2009-10 seasonal influenza vaccines administered 7 weeks apart. Group (1) received 2 doses of live attenuated intranasal influenza vaccine (LAIV); Group (2) received 2 doses of inactivated influenza vaccine (IIV); Group (3) received LAIV then IIV; Group (4) received IIV then LAIV. A range of assays for avian H5N1, 2009 pandemic H1N1, and seasonal vaccine influenza strains were performed on blood and nasal wash samples collected pre-vaccine and 2 and 4 weeks after each dose, and the percentage of cytokine-producing cells 14 days after each dose was compared with baseline. As expected, subjects receiving IIV had prompt serological responses to vaccine strains, which were not measureable in subjects receiving intranasal vaccine. Two of 8 subjects in Group 3 demonstrated cross-reactivity against H5N1 and pandemic H1N1 2009, as well as against the seasonal vaccine strains. All vaccine regimens were safe and well tolerated. In this pilot study a prime-boost regimen of seasonal influenza vaccines gave laboratory evidence of cross-protection against both avian and pandemic influenza in a quarter of subjects. This strategy may be a useful adjunct in the event of a new

influenza pandemic while a specific vaccine is being developed. Further work is needed to study the immune response to influenza vaccines in the expectation that a universal influenza vaccine can eventually be developed.

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HUMAN INFLUENZA SENTINEL SURVEILLANCE IN REMOTE BORDER POPULATIONS IN WESTERN CAMBODIA

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Seasonal, pandemic H1N1 and avian influenza viruses have been reported from Cambodia. However, despite significant cross-border population movement, influenza surveillance data on the Cambodian side of the Thai-Cambodian border is limited. In May 2010, the Armed Forces Research Institute of Medical Sciences (AFRIMS) initiated an influenza sentinel surveillance study in Western Cambodia with the Cambodian Communicable Disease Control (CDC) Department and Institut Pasteur du Cambodge. Epidemiological data and nasal and throat specimens were collected from outpatients who presented with influenza-like-illness (ILI - fever > 38°C and cough or sore throat) at sentinel health facilities in Battambang and Oddar Meanchey provinces. Real-time PCR was performed to distinguish influenza A and B, and positive samples were subtyped. Influenza-negative samples were analyzed for alternate respiratory pathogens by inoculation onto MDCK cells and detection by immunofluorescence test. Of 82 ILI patients recruited between May 2010 and May 2011, 13 specimens tested positive for influenza by real-time RT-PCR between August and October 2010, of which 12 were influenza A and 1 influenza B. Subtyping of influenza A viruses detected 9 (75%) pandemic influenza A/H1N1(2009) and 3 (25%) influenza A/H3N2, yet no influenza A/H5N1 or seasonal influenza A/H1N1. Among flu-negative samples tested to date, only 1 non-influenza respiratory pathogen (parainfluenzavirus type 3) was detected. Genomic data, along with oseltamivir susceptibility data from influenza A isolates will be presented. In newly established sentinel surveillance sites along the Thai-Cambodia border, influenza virus was relatively infrequent among patients presenting with ILI, with pandemic influenza A/H1N1(2009) being the most common virus identified. Highly Pathogenic Avian Influenza (HPAI) virus H5N1 was not detected, and few common respiratory pathogens were isolated from influenza-negative samples.

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SEASONAL LEVELS OF INDOOR RESPIRABLE PARTICULATE MATTER IN A LOW-INCOME COMMUNITY IN URBAN BANGLADESH

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Acute lower respiratory infections (ALRI) are the leading cause of death in children <5 years of age in Bangladesh and indoor exposure to respirable particulate matter has been repeatedly associated with increased risk of ALRI in young children. This study aimed to describe air pollution exposures in households with young children in urban Dhaka and seasonal variation of this exposure. Two hundred and thirty-five households with children aged <18 months in an urban, low-income neighborhood were enrolled. Particulate matter approximately 2.5 microns in diameter ($PM_{2.5}$) was measured in the child's sleeping space for one 24-hour period each month using a portable monitoring device during May 2009 - April 2010. We calculated the arithmetic mean concentration of $PM_{2.5}$ among all households by month and investigated differences in mean concentrations by the type of cookstove used. We compared these values to WHO recommended daily mean values of 25 μg/m^3. We estimated the average number of hours per day that $PM_{2.5}$ levels exceeded 100, 250, 500, and 1000 μg/m^3. Seventeen percent of 235 households reported burning biomass as their primary cooking fuel; all other households cooked with natural gas or electricity. Mean $PM_{2.5}$ concentrations were 200 μg/m^3 but were significantly higher in households that burned biomass compared to cleaner fuels (265 vs 187μg/m^3, p=0.004). Biomass users had higher concentrations during all seasons. The highest mean concentrations were observed during winter for both biomass and cleaner fuel users (467 and 363 μg/m^3). Overall, $PM_{2.5}$ concentrations were above 100 μg/m^3 for approximately 5 hours and 30 minutes per day, but this increased to >10 hours per day during the winter. The hours that concentrations exceeded 250, 500 and 1000 μg/m^3 all doubled during winter compared to the yearly average. $PM_{2.5}$ concentrations exceeded 1000 μg/m^3 for more than an hour a day during the winter. Our study suggests that 24-hour mean $PM_{2.5}$ concentrations are frequently 10 times higher than the WHO recommended 24-hour mean in this low-income urban neighborhood and may contribute to increased risk of ALRI for children in this community. Burning biomass for cooking was associated with significantly higher $PM_{2.5}$ concentrations but even households that burned cleaner fuels were highly polluted, particularly during the winter. In this setting, high indoor $PM_{2.5}$ concentrations are not fully explained by burning biomass.

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DELAY IN DIAGNOSIS OF TB PATIENTS IN ADEN GOVERNORATE, YEMEN: EVALUATION OF THE HEALTH SECTOR PERFORMANCE

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Yemen has adopted the DOTS strategy to control TB since it was launched by the WHO STOP TB initiative in 1995. The program functions under the supervision of the public health sector without involvement of the private sector. Aside from being not involved, doctors in the private sector may also resist to follow the WHO's DOTS guidelines which have been proved highly effective in managing sputum-smear positive (SS+) TB patients. We designed a retrospective cross-sectional study to compare the delay in diagnosis and treatment of SS+ TB patients between the public and private health sectors. A total of 171 new SS+ patients aged 15 years or older registered in Aden governorate over a three-quarter period (Oct, 2008-June, 2009) have been interviewed using a pre-designed and tested semi-structured questionnaire to collect the required data including patient's care-seeking behavior. Written informed consents have been obtained from all patients who were willing to participate; data has been analyzed using SPSS 18.0 and STATA v9. 112 (65.5%) males vs. 59 (34.5%) females with a male to female ratio of 1.9:1, mean age of 35.9 (±15.18) years, 161(94.2%) were Yemeni nationals vs. 9 (5.8%) Somalian and 1 (0.6%) Ethiopian refugees. 146 (85.4%) patients were residents in Aden vs. 25 (14.6%) from other governorates, 23 (13.5%) patients had DM and 62 (36.3%) patients had a positive family history of TB. Mean patient delay (appearance of symptoms until seeking care) was 36.2(±67.58) vs. 37.24(±123.4) days; mean diagnostic delay (patient's first contact with the health provider until TB diagnosis) was 9.8 (±15) vs. 19.45(±42) days; and a mean treatment delay (diagnosis until start of treatment) of 1.56(±1.6) vs. 1.56(±0.86) days for public and private sectors respectively. After logarithmic transformation of the three means, only the mean diagnostic

delay was significantly longer in the private sector than in the public sector (one-way ANOVA, F= 5.11, p<0.02). Not only emphasizing the importance of adhering to the WHO guidelines for DOTS strategy; these findings necessitate the urgent integration of the private health sector into the DOTS strategy to reduce the gap between the public and private health sectors and to positively strengthen the activities directed to control TB in Yemen.

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MOLECULAR ANTIVIRAL SUSCEPTIBILITY TESTING OF SEASONAL INFLUENZA A VIRUS ISOLATES OBTAINED IN KENYA IN THE YEAR 2008-2009

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Antivirals play an important role in treatment and prevention of severe influenza infections. Amantidine and remantidine inhibit M2 protein of influenza A while oseltamivir and zanamivir inhibit NA of influenza A and B. M2 protein mediates influx of protons through the viral lipid membrane causing dissociation of the virus during virus entry. Binding of M2 inhibitors to M2 inhibits viral genome uncoating and RNP import into the nucleus. NA catalyzes removal of terminal sialic acid residues from viral and cellular glycoconjugates to facilitate virus release. NA also helps virus spread through circulation by removing sialic acids from cell surfaces. NA inhibitors interfere with release of progeny virus from infected cells preventing infection of new cells and halting the spread of infection. Mutations in M2 and NA proteins underpin these resistances at the molecular level. H274Y (H275 in NA1) change in the NA protein alters drug binding resulting in oseltamivir resistance. S31N substitution in the M2 domain determines antiviral resistance to M2 inhibitors. We investigated genetic characteristics of NA and M2 genes of seasonal influenza A virus isolated in Kenya in 2008-2009 in relation to antiviral resistance. Nasopharyngeal specimen from out patients ≥ 2 months old were screened by rtRT-PCR. Positive specimens were inoculated on MDCK cells followed by RNA extraction and amplification of M and NA genes. We sequenced 12 influenza A(H1N1) and 36 influenza A(H3N2) M and NA genes. 58% of influenza A(H1N1) viruses had H275Y mutation but none had S31N change. All H3N2 strains had the S31N mutation in M2 protein. All H3N2 strains lacked H274Y mutation. These results conform to the global picture regarding influenza antiviral activity during the period. In conclusion, genotypic data obtained here demonstrate antiviral resistance in seasonal influenza A viruses isolated in Kenya in 2008-2009 despite lack of widespread antiviral use. Our results emphasize the unpredictable nature of influenza viruses and need for continued surveillance of drug resistance patterns globally.

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A QUALITY ASSESSMENT TOOL FOR TUBERCULOSIS CONTROL ACTIVITIES IN RESOURCE LIMITED SETTINGS

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Tuberculosis (TB) is a significant problem, infecting nearly 9 million new patients per year and killing about 2 million a year. The primary means with which to affect TB globally are to decrease transmission locally, mainly by effective identification, diagnosis, and treatment of infectious TB patients. Therefore, quality assurance of TB control efforts at the local level is essential. This study describes the creation of a data extraction tool for retrospective chart review based on the *International Standards for TB Care, 2009* for the assessment of TB control programs located in resource limited settings. The tool was field tested at a rural mission hospital in central Kenya. Results highlight multiple areas of excellence in TB care such as patient retention, treatment completion, and care of HIV/TB co-infected patients. Quality improvement interventions might best be focused on smear negative diagnosis and follow up sputum smears for smear positive patients. The process prompted revision of the tool to clarify questions and answers. The final product is a good tool to collect data for use in quality assessment and improvement of local TB control programs in resource limited settings.

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GENOME-WIDE ASSOCIATION STUDIES OF TUBERCULOSIS AND LEPROSY SUSCEPTIBILITY IDENTIFY COMMON PATHWAYS INVOLVED IN BOTH DISEASES

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Tuberculosis (TB) and leprosy cause significant worldwide morbidity and mortality, predominately in resource poor settings across Africa and Southeast Asia. Although TB and leprosy represent the largest burden of mycobacterial disease in humans, relatively few studies have attempted to analyze human susceptibility loci which associate with predisposition to both of these diseases. TB and leprosy are significantly divergent in terms of phenotypic presentation, however, the causative agents *Mycobacterium tuberculosis* (TB) and *Mycobacterium leprae* (leprosy) share a common origin, and it is likely that the human innate immune system detects these pathogens using shared Toll-like receptor signaling. Our group has recently published genome-wide studies on both tuberculosis and leprosy and we here identify genes and pathways that associate with human genetic susceptibility to both diseases using data from these genome-wide studies. Aggregate gene based association statistic for mycobacterial susceptibility was computed using data from tuberculosis and leprosy susceptibility studies and these results were further carried forward for pathway analysis. Significant associations were observed between the *CYP11B1/2*, *CTSB/FDFT1*, *CYP26A1*, *AGER* and susceptibility to common mycobacterial diseases (P_{best}=5.1x10^{-6}). Pathway analysis implicates the lipid and steroid hormone metabolism pathways (P_{best}=2.2x10^{-5}). In summary, we find evidence that lipid and steroid hormone metabolism pathways play an important role in susceptibility to both tuberculosis and leprosy. We are now initiating a genome-wide study of Buruli ulcer (BU), caused by *Mycobacterium ulcercans*, in African populations to compare overlapping susceptibility loci between these three mycobacterial diseases. These studies have the power to elucidate novel molecular pathways which represent potential targets for pharmacological intervention for future treatment of mycobacterial diseases.

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TOWARDS GLOBAL CHARACTERIZATION OF ENVIRONMENTAL AND CLIMATIC DETERMINANTS FOR SEASONAL INFLUENZA

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Recent experimental studies have established the role of factors such as humidity and temperature in influenza transmission. Historically, spatiotemporal spread of influenza has been observed to follow a

latitudinal gradient, with environmental and climatic factors. With the capability of pandemic and seasonal influenza to spread rapidly worldwide - it is even more important to understand how climactic and environmental factors affect the efficiency of influenza transmission in different parts of the world so as to enhance multilateral efforts for prevention and control. We use NASA satellite-derived data in various population centers throughout the world to obtain indicators including land surface temperature and precipitation, and ground station measurements such as relative humidity. Trends of influenza-like illnesses in these same population centers were also identified. We further developed linear and non-linear models, in order to determine the dominant factors contributing to influenza transmission. Since different countries have varying completeness of data and different surveillance systems, we used a range of models, including time series regression and neural network. The first phase of our study included countries in North and Central America, and Northern Europe, encompassing both sub-tropical and temperate climate. Our results show that measures for rainfall, temperature, relative humidity and solar radiation contribute to influenza dynamics. About 60% of influenza variability in temperate regions can be accounted by these factors. Whereas in sub-tropical region, previous number of influenza cases are additionally needed as a determinant. Our best fit models can predict influenza cases with more than 75% accuracy. Our study may help develop better ability to forecast influenza activity worldwide. The methods in turn, can be integrated into surveillance system as an early warning system, for both seasonal and pandemic influenza.

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BORDER CONTROL MEASURES AND TERRITORIAL SURVEILLANCE IN AMERICAN SAMOA FOR THE 2009 H1N1 INFLUENZA PANDEMIC

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Our objective is to describe the disease prevention, case identification, and treatment approaches of the American Samoan Unified Health Command during phase one of the 2009 H1N1 Influenza Pandemic. A retrospective review of public health surveillance records, hospital infection control records, and event after-action reports was completed. Descriptive statistics were used to evaluate data. Border surveillance measures were in effect from May 3 to June 8 when the first confirmed H1N1 case was documented in the territory. Health officials met all incoming aircraft and sea vessels at territory ports of entry. Surveillance forms were requested from each passenger, and passive screening techniques for illness identification were employed. Cases of influenza-like-illness were further investigated at a local clinic and suspected travelers were placed in community isolation. Greater than 3200 man hours were documented during surveillance activities. Hospital surveillance data from May 3 to July 31, 2009 was collected. Ninety-nine influenza swabs sets were collected. On sight rapid testing demonstrated 19.2% (n=19) Influenza A + samples, and 37.4% (n=37) H1N1+ samples by confirmatory testing off-island. The sensitivity and specificity of this screening strategy were 30.5% and 87.3% respectively (PPV 57.9%, NPV 68.7%). A true positive rate of 11.1% and false negative rate 25.3% further complicated medical decision-making based on rapid testing alone. Eight percent of cases were suspected concomitant seasonal influenza A strains. No fatalities were reported. In conclusion, a unified health command structure was effective in responding to this emergency. Response planning was appropriate and rapidly implemented. Border control surveillance efforts were largely ineffective. Case identification and medical treatment protocols were hindered by remote locale. Despite the challenges discussed, no fatalities were reported.

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AN EIGHT ANTIGEN MULTIPLEX ELISA DERIVED FROM A FULL PROTEOME SCREEN FOR ACCURATELY DETECTING ACTIVE TUBERCULOSIS

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Developing accurate serological assays for the detection of active tuberculosis has been difficult, because no single antigen has been found that is recognized by serum antibodies in every tuberculosis patient. It has become increasingly clear that there is no "magic bullet," and that an accurate serological test for active TB will require a panel of antigens and a multiplexed approach. In an effort to identify a collection of antigens from which to develop a multiple antigen serological test, we developed a full proteome microarray made up of approximately 4000 different M. tuberculosis proteins. The array was probed with 257 culture-confirmed TB cases and 307 non-TB controls from 9 different clinics in Africa, Asia, South America and Canada. Background reactivity against Mycobacterium was noted in all non-TB subjects with the highest in Africa followed by Asia, South America and Canada. Differentially reactive antigens were identified that are significantly more reactive in the TB cases than controls. Smear positive cases are more reactive to these antigens than smear negative TB cases. ELISA assays using eight of the antigens together in a multiplex assay could differentiate between TB cases and controls with 91% sensitivity and 83% specificity. These results provide a framework for understanding the humoral immune response to M. tuberculosis infection and for developing more accurate serodiagnostic tests.

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DIAGNOSIS AND MOLECULAR CHARACTERIZATION OF CRYPTOSPORIDIOSIS AND CYCLOSPOROSIS ON INFECTED CHILDREN UNDER FIVE YEARS OLD FROM A NGÖBE-BUGLE COMMUNITY IN WESTERN PANAMA

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The Ngöbe-Bugle is an ethnic group that is located in several rural regions of Western Panama, where the sanitary conditions are frequently very poor. These conditions are favorable for the transmission of several intestinal parasitic infections mainly in children. *Cryptosporidium* sp. and *Cyclospora cayetanensis* are intestinal coccidian that causes diarrhea and malnutrition in children. In Panama, few studies have been conducted to establish the prevalence of intestinal coccidian particularly in Amerindian communities. The aim of this study was to determine the prevalence and genetic characteristics of *Cryptosporidium* spp. and *C. cayetanensis* in 236 children under five years old, from two Ngöbe- Bugle communities. Stool samples were concentrated by acetate ethyl formol technique and the sediment stained with Kinyoun´s stain. *Cryptosporidium* infections were confirmed/characterized by a PCR-RFLP analysis using the SSU rRNA gene as a molecular marker. Further genetic diversity of *Cryptosporidium hominis* positives samples were assessed by sequence analysis of the GP 60 gene. The results revealed that 75/236 (31.7%) evaluated samples were positive for *Ascaris lumbricoides*, 64/236 (27%) for *Giardia lamblia*, 22/236 (9.3%) for hookworms, 11/236 (4.7%), for *Trichuris trichiura,* 15/236 (6.3%) for *Entamoeba histolytica/* E. dispar complex and 97/236 (41%) for non pathogenic intestinal protozoa. The frequency found for intestinal coccidian was of 4.6% (11/236), 2.5% (6 /236) for *Cryptosporidium* spp, 1.3% (3/236) for *Cryptosporidium hominis,* 0.8% (2/236) and 2.2% (5/236) for *C. cayetanensis.* In addition *C. hominis* subtype Ie was confirmed in one sample by the sequencing analysis of the GP 60 gene. This study demonstrates a high prevalence of intestinal

parasites, including coccidian, in the evaluated Ngöbe- Bugle children. Furthers studies are necessary to establish the role of these parasites in the general health status of this ethnic group.

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CYCLOSPORA CAYETANENSIS IN A PEDIATRIC HOSPITAL IN MORELIA, MÉXICO

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Cyclospora cayetanensis affects immunocompetent and immunocompromised individuals and has been associated with food and waterborne gastrointestinal illness characterized by watery and persistent diarrhea and abdominal pain. Cyclosporiasis has been associated with traveler's diarrhea and the consumption of fresh fruits and vegetables. In the present study, stool samples from 8,877 children were examined for ova and parasites at the Pediatric Hospital of Morelia from 2000 to 2009. Sixty children (0.67%) had *Cyclospora* in their stools. Diarrhea (33.3%), abdominal pain (31.6%), and vomiting (15%) were the most frequent symptoms. Most of the cases (93.3%) were observed between June and August, the rainy season. In 45 children, *Cyclospora* was the only parasitic pathogen detected, while 8 children (13%) also had commensal parasites with an overall co-infection in 15 (25%) children. Our findings suggest that *C. cayetanensis*, a parasite with unique geographical distribution, may be endemic in Michoacán, showing a characteristically seasonal pattern.

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EVOLUTION OF SEROLOGICAL TESTS OF TOXOPLASMOSIS IN PREGNANT WOMEN FROM SENEGAL

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Toxoplasmosis is a well-known disease in Europe where its epidemiology was well studied in many countries. In many African countries including Senegal, toxoplasmosis is not subject of a real understanding. The objective of this study is to reassess the toxoplasmosis antibodies prevalence among pregnant women during pregnancy medical surveillance. The test has been performed in 941 pregnant women at the laboratory of parasitology and mycology at Le Dantec teaching hospital from 2002 to 2006. immunoenzymatic method in solid phase has been used. To accomplish this evaluation, two serological tests (S1 and S2), using venous blood at 3 weeks of interval, are carried out among these pregnant women. The second serology will allow confirming a toxoplasmosis from a immune response, or a non specific antibody fixation. From the 941 patients tested, we found a prevalence of 7,7% and 0% for (IgM+IgG-) respectively at serology S1 and S2; 23,3% and 24,3% for (IgM-IgG+), 11,3% and 10,2% for (IgM+IgG+). 34,5% of pregnant women present toxoplasmosis antibody. These data confirm the presence of toxoplasmosis among pregnant women in Dakar

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TOXOPLASMOSIS HOSPITALIZATION TRENDS IN THE UNITED STATES, 1993-2008

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Trends for toxoplasmosis-related hospitalizations have not been reported recently in the United States. Toxoplasmosis-related hospitalizations often occur in persons with HIV infection and other causes of advanced immunosuppression. Using the National Inpatient Sample (NIS), a component of the Healthcare Cost and Utilization Project, we examined trends in toxoplasmosis-related hospitalizations by HIV infection status from 1993 through 2008. The NIS is designed to represent a 20% sample of U.S. community hospitals and includes information on up to 8 million hospital discharges per year from approximately 1,000 hospitals. The NIS is weighted to produce national estimates. States included in the NIS increased from 17 in 1993 to 40 in 2007. ICD 9 codes 130-130.9 were used for toxoplasmosis and 042-044/795.8/V08 for HIV infection. Estimated HIV-associated toxoplasmosis hospitalizations increased from 9,395 (95% confidence limits [CL] 6,902, 11,889) in 1993 to 10,583 (95% CL 7,628, 13,537) in 1995 then dropped sharply to 3,643 (95% CL 2801, 4485) in 2001 with similar levels thereafter. The rate of HIV-associated toxoplasmosis hospitalizations among all HIV-related hospitalizations decreased from 3.33% (95% CL 3.18%, 3.49%) in 1993 to 1.25% (95% CL 1.15%, 1.35%) in 2008. Non-HIV associated toxoplasmosis hospitalizations remained relatively constant from 1993-2008 in approximately the 400-800 range (associated percents were.0020% [95% CL.0017,.0024] and.0015% [95% CL.0012,.0018], respectively). HIV-associated toxoplasmosis hospitalizations dropped markedly after 1995 when highly active antiretroviral therapy for HIV infection was introduced, however, hospitalizations have decreased relatively little after 2000 suggesting that some HIV-infected persons are being tested late or antiretroviral therapy is failing due to resistance, poor compliance, or other reasons. The number of non-HIV associated toxoplasmosis hospitalizations has remained more stable.

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HIGH *CRYPTOSPORIDIUM PARVUM* ANTI-IGG SEROPREVALANCE AMONG HIV-POSITIVE ADULTS IN LIMPOPO AND OTHER REGIONS OF SOUTH AFRICA

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Cryptosporidium spp. are common causes of persistent diarrhea associated with stunting and likely cognitive impairments in malnourished children, and can be life threatening in severely immunocompromised hosts. Literature remains sparse, however, regarding region-specific exposures, and host mechanisms conferring immunity are unclear. A cross-sectional study was conducted to determine the seroprevalance of *Cryptosporidium parvum* in the Limpopo region in South Africa. Banked frozen plasma from 194 HIV-positive adults (11-69 years of age) in Limpopo and neighboring provinces, and recently collected plasma from 58 University of Venda (UNIVEN) healthy volunteers were screened for *C. parvum* IgG antibody with a crude *C. parvum* antigen (Iowa Strain, Waterborne, Inc.) ELISA. Using a previously defined cut-off value of 1.8 times the internal negative control optical density (OD) (mean OD negative control=0.186±0.05) on each plate, the seroprevalence was 70.6% among HIV-positive patients and 32.8% among UNIVEN students (P<0.001). Seroprevalence was high throughout Limpopo as well as neighboring provinces (50-100%). Anti-*Cryptosporidium* IgG was detected in 29 of 44 (65.9%) patients with advanced HIV disease (AIDS or WHO Stage 4). An age-matched comparison between the two groups showed increased risk for anti-*C. parvum* IgG in those with HIV (OR=2.99; 95% CI: 1.48-6.04). In a parallel pilot assay, among twelve of the healthy UNIVEN students, there was no correlation seen between seropositivity and IFN-γ released following whole blood stimulation (QuantiFERON®-CMV**,** Cellestis) with an identical crude *C. parvum* antigen (R^2=0.032). The sustained *C. parvum* IgG antibody responses seen throughout adulthood imply ongoing exposures in rural regions of South Africa. The discrepant ELISA and IFN-γ release assay results warrant further field investigations and laboratory models to elucidate the host immune response to this ubiquitous pathogen.

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DEVELOPMENT OF A NESTED PCR PROTOCOL BASED ON INTERNAL TRANSCRIBED SPACER (ITS) REGION FOR RAPID DETECTION OF HUMAN-PATHOGENIC *CYCLOSPORA CAYETANENSIS* PARASITES

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Cyclospora cayetanensis is a human-pathogenic coccidian parasite causing acute diarrheal disease. Although it is endemic to tropical countries, it has been reported from several geographic regions worldwide. This parasite has been identified as the cause of several foodborne outbreaks in the United States and Canada associated with imported produce, predominantly raspberries. Multilocus genetic characterizations have proven to be advantageous for tracing diminutive genetic variations in several human-pathogenic organisms with low natural genetic diversity including apicomplexan parasites. In our previous study we have examined the 70 kDa heat shock protein (HSP70) gene *C. cayetanensis.* In this study we have described the development of a nested PCR protocol based on the internal transcribed spacer (ITS) region for rapid detection of *C. cayetanensis* parasites in humans. This newly developed nested protocol was tested and authenticated by PCR amplification and nucleotide sequencing. Eighteen human *C. cayetanensis* isolates from three endemic regions including Nepal, Mexico, and Peru were PCR amplified using this ITS primer set. Analysis of the generated ITS nucleotide sequences revealed the *C. cayetanensis* parasites to be a genetically distinct species within the genus *Cyclospora*. This newly developed ITS-based nested PCR protocol provides another useful genetic marker for rapid detection of *C. cayetanensis* parasite in future.

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SEROPREVALENCE OF ANTIBODIES TO *TOXOPLASMA GONDII* IN MALI

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The protozoan parasite *Toxoplasma gondii* is widely distributed throughout the world and its prevalence varies considerably by countries based on local behavioral and environmental risk factors. Toxoplasmosis infection is normally subclinical in immunocompetent adolescents and adults, but *T. gondii* is a prominent opportunistic pathogen associated with AIDS. Congenital infection can have severe consequences on fetus and infants. We conducted a serological survey on existing 650 serum samples collected for malaria studies to assess the seroprevalence of toxoplasmosis in an urban and a rural setting of Mali. Antibody levels were measured using a modified agglutination test assay. A seroprevalence of 24.7% and 26.8% % was observed in adults from the urban (Bamako) and rural setting (Kolle) respectively. No significant difference was observed between the seroprevalence in men *vs.* women. In the rural village of Kolle, seroprevalence rose from 0% in infants (< 1 year) to 0.8% (1-5 yr), 2.7% (6-10 yr), 11.3% (11-15 yr), and 26.8% (>15). The seroprevalence was significantly different between children <10 and the 11-15 yr age group ($p < 10^{-3}$), and between 11-15 and adults ($p = 0.04$). IgG Serum titers in the population increased in parallel with seroprevalence. Modeling the observed age distribution suggests a seroconversion rate of ~2%/yr. This study suggests that congenital toxoplasmosis may be an under-studied public health concern in Mali.

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CLONORCHIASIS: AN EMERGING AND UNDERESTIMATED FOODBORNE TREMATODIASIS IN CHINA

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Clonorchiasis, caused by the oriental liverfluke, *Clonorchis sinensis,* is a major foodborne trematodiasis endemic to parts of Asia including China, Korea, Taiwan, Vietnam, and a small part of Russia neighboring north-eastern China. A conservative estimate suggests that at least 35 million people are infected by this parasite in these regions. In China, despite archaeological evidence suggesting that human infections could be traced back to at least 2,300 years ago, distribution of endemic areas and population affected by the disease was unclear until the first national survey in 1990, indicating that infected people were distributed in 22 provinces/cities with an overall prevalence of infection 0.31% and highest infection (1.82%) in Guangdong Province. In the second national survey during 2002-2004, however, anoverall 75% increase in human prevalence of infections was observed compared to the first national survey, with particularly significant increases in Guangdong, Guangxi, and Jilin where 182%, 164%, and 630% increases were seen, respectively.In Guangdong Province, the recent survey indicated that there are 63 endemic counties where human infections are reported to range between 0.2% and 50.3% at the county/city level. Over 5 million people are estimated to be infected in Guangdong. Although the survey indicated that consuming raw or undercooked fish infected by the parasite and poor sanitation causing fish pond contamination are the main risk factors, little is known about the public health impact (e.g. disease burden) in these endemic areas, transmission pathways beyond the risk factors, and ecology of the parasite and its intermediate hosts. Research needs and challenges in controlling clonorchiasis in China are discussed.

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EVALUATION OF THE EFFICACY OF OXFENDAZOLE AGAINST *FASCIOLA HEPATICA*

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Fasciola hepatica is the most important liver parasitic disease of ruminants in wide areas of the world, with economic implications due to reduced milk production, meat or wool. Fasciolosis is also a zoonotic disease causing considerable morbidity by damages in the human liver and biliary system. Although numerous treatments have been used in the past on humans and animals, most were poorly effective and now the drug of choice is triclabendazole. Triclabendazole resistance has already been demonstrated in small ruminants in different countries. A potential single dose alternative treatment, oxfendazole, has not yet been evaluated for fasciolasis. In this study, we evaluated the efficacy of oxfendazole in 40 adult sheep naturally infected with *F. hepatica*. Sheep belonged to a community in an endemic area to fasciolasis. All the animals were screened for *Fasciola* eggs one day before the beginning of the study (day 0) by sedimentation method to confirm that their fecal egg counts was higher than 2 eggs per gram (*epg*). The animals were randomly allocated in two groups of 20 sheep each. The first group was left untreated (control), while the second group (treatment) was treated orally with oxfendazole at a single dose of 30 mg/kg of body weight. Fecal samples were taken from each animal 10 days after the treatment, and the number of *Fasciola* eggs was determined with the same method. In the day 0, all animals (two groups) had on average 4.65 (2-28) *epg.* Ten days after

treatment, the control group had 7.5 (2-37) *epg*, while and the treatment group had 0 *epg*. A single dose of 30 mg/k of oxfendazole is safe and 100% efficacious in sheep infected with *F. hepatica*.

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FIVE SPECIES OF ECHINOSTOMES RECOVERED FROM HUMANS IN KHAMMOUANE PROVINCE, LAO PDR

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Echinostomes (= family Echinostomatidae) are intestinal trematodes of humans and animals, and can cause severe epigastric or abdominal pain accompanied by diarrhea, easy fatigue, malnutrition, and rarely mortality in human infections. In the present study, 5 species of echinostome flukes were recovered after praziquantel treatment of 9 people living along the Mekong River in Lao Peoples' Democratic Republic (Lao PDR). The surveyed areas were riverside (tributaries of the Mekong River) villages in Khammouane Province. A total of 1,242 fecal samples were collected and examined using the Kato-Katz thick smear technique. Echinostome eggs, species undetermined, were detected in 9 people (0.67%), i.e., 6 male and 3 female patients. These egg positive people were given a single oral dose of 10-20 mg/kg praziquantel and purged with 20-30 g $MgSO_4$. Worms were collected from their diarrheic stools, fixed in 10% formalin, stained with acetocarmine, and morphologically identified. A total of 52 echinostome specimens were recovered. They consisted of 5 species, including 6 specimens of *Echinostoma revolutum*, 5 of *Echinostoma macrorchis*, 4 of *Euparyphium murinum*, 2 of *Artyfechinostomum malayanum*, and 31 of *Echinochasmus japonicus*. All these echinosotme species are reported for the first time in Lao PDR. *E. murinum* turned out to be a new zoonotic parasite so far as the world literature are concerned. We report echinostomiasis as one of the endemic trematode infections among villagers of Khammouane Province, Lao PDR.

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SEROLOGICAL DIAGNOSIS OF NORTH AMERICAN PARAGONIMIASIS BY WESTERN BLOT WITH *PARAGONIMUS KELLICOTTI* ADULT WORM ANTIGEN

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Human paragonimiasis is an emerging disease in Missouri. 12 cases have been diagnosed since 2006, and many patients presented with serious illnesses. The infection is caused by *Paragominus kellicotti* (Pk), which is highly prevalent in crayfish in Missouri rivers and streams. The purpose of this study was to develop and evaluate an antibody serology test for diagnosis of Pk infections. We infected gerbils with Pk metacercariae and recovered adult parasites 6 wks later. An adult worm antigen extract was used to detect IgG antibodies by Western blot on 4-12% gradient gels. The test was evaluated with sera from 30 healthy Americans (HA), 39 sera from patients with other helminth infections (OHI, *Strongyloides, Schistosoma, Fasciola, Echinococcus*), 7 sera from patients infected with *P. westermani* (Pw, Philippines), and 10 from patients with proven *P. kellicotti* infection (Pk, confirmed by CDC Western blot using *P. westermani* antigen or by recovery of eggs from sputum/BAL). Two other sera were tested from 2 suspected Pk (sPk) cases that were negative by Western blot at CDC. All 10 Pk sera and both sPk sera contained antibodies to an antigen at 36 kDa and a doublet at 24/26 kDa. Six of the 10 PK sera also recognized an antigen at 8 kDA. Some sera also labeled other antigens at 4, 12, 14, 44, 47, 49, 55 and 62 kDa. All 7 Pw sera labeled the 36kDa antigen, but only 2 labeled the 24/26 kDa antigens, and 6 labeled the 8 kDa antigen. Several HA and OHI sera weakly labeled antigens at 14, 25, 29, 44, 47, 49, 55 and 62 kDA, but none labeled antigens at 36, 24/26, or 8Kda. Based on these results, we consider sera that label either the 36 kDa or the 24/26 kDA bands to be positive for antibodies to *P. kellicotti*. Antibody responses were significantly reduced from baseline in sera from two patients that were collected 6 months after praziquantel therapy. Thus, the Pk Western blot appears to be highly sensitive and specific for diagnosis of paragonimiasis, and it may also be useful as a test of cure. Pk antigen appears to be superior to Pw antigen for diagnosing Pk infections.

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SYNERGISTIC REVERSAL OF PATHOLOGY DUE TO CHRONIC *SCHISTOSOMIASIS MANSONI* BY PRAZIQUANTEL AND DT_{390}-IL-18 TARGETED PLASMID IMMUNOTOXIN IN EXPERIMENTAL BALB/C MICE

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Patient with chronic schistosomiasis often fail to resolve hepatic fibrosis after antehelminthic treatment. DT_{390}-IL18 immunotoxin suppresses immunopathology in schistosomiasis by destroying antigen activated APCs and T cells. Therefore, we studied the synergistic effects of DT_{390}-IL-18 and Praziquantel (PZQ) on the resolution of pathology due to chronic *Schistosoma mansoni* infection in mice. 13 weeks after exposure to 25 *S. mansoni* cercariae, mice were treated with Placebo, PZQ, DT_{390}-IL-18, or PZQ+DT_{390}-IL-18. Pathology was assessed by granuloma size, hepatic weight, hepatic proline, hydroxyproline, collagen I and III and fibronectin content and infiltrating cells phenotypes. Mice that received PZQ+DT_{390}-IL-18 had a very significant increase in the rate of resolution of hepatic fibrosis (+65%) when compared to placebo treated control, PZQ (+35%) or DT_{390}-IL-18 (+23%) alone. Similar synergistic suppression of $CD4^+$, IL-18 αR, Th1 and Th2 cells in hepatic granulomas and the induction of apoptosis were also observed. Therefore, the resolution of immunopathology in mice with chronic schistosomiasis is enhanced with a combination of chemotherapy and immunotherapy. This therapeutic approach could be of potential use in humans demonstrating pathology due to chronic hepatosplenic schistosomiasis.

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CPG-ODN REPRESENTS AN ALTERNATIVE ADJUVANT TO BE USED IN A VACCINE FORMULATION AGAINST SCHISTOSOMIASIS

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Currently schistosomiasis control is mainly based on chemotherapy, but in spite of decades of mass treatment, the number of infected people remains constant. A vaccine that induces even a partial reduction in worm burdens could reduce pathology and limit parasite transmission. The surface of the *Schistosoma mansoni* schistosomula (Smteg) is an important target for host immune system attack since it represents the interface between host and parasite and thus is a potential candidate

for vaccine development, recently we have shown that Smteg is able to activate dendritic cells *in vitro* and also can induce 43-48% protection in mice when in association with Freund`s adjuvant. In this study we evaluated the ability of different adjuvants (alum or alum +CpG) in association with Smteg to induce protection in a three dose immunization protocol in mice. Thirty days after the third dose, mice were infected and 50 days pos-infection mice were perfused. During the immunization, blood samples were collected for the ELISA assay. A stool examination was also performed. The number of eggs in the liver and intestine wall was determined. The profile of the immune response induced by each formulation was determined by cytokine measurement and immune cells characterization. In the group of mice immunized with Smteg alone or with alum, no protection was observed, however immunization with Smteg + alum+ CpG-ODN were able to induce a 43-51% reduction on adult worm burden; 35 % in the number of eggs/g in the liver and intestine; 54% in the number of eggs/gram of faeces. The protective immunity observed in Smteg/alum/CpG-ODN group was associated with a increase production of specific IgG2c antibodies, significant production of IFN-γ and IL-10 by CD4+ cells, activation of CD4+ cells, and increased expression of CD86 in F4/80+ cells. These results not only confirm Smteg antigens as potential candidates to be used in a vaccine formulation but also indicate an alternative adjuvant and the immune response associated with protection.

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HISTONE MODIFYING ENZYMES (HMES) OF *SCHISTOSOMA MANSONI* AND *S. JAPONICUM*

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Schistosomes infect over 200 million individuals in more than 75 countries in tropical or subtropical regions and cause more than 280,000 deaths annually. The genomes and predicted proteome sequences of *Schistosoma mansoni* and *S. japonicum* were recently published providing an opportunity to identify new drug candidates. Histone modifying enzymes (HMEs) are major players in the regulation of chromatin epigenetic modifications. Furthermore, aberrant epigenetic states are often associated with cancer, leading to great interest in HMEs as therapeutic targets. In order to choose potential drug targets for further study, we have characterized all enzymes involved in either acetylation or methylation histone modifications: histone deacetylases (HDAC), histone acetyltransferases (HAT), histone methyltransferases (HMT), and histone demethylases (HDM). We analyzed the *S. mansoni* and *S. japonicum* predicted proteomes to identify and classify these HME families through computational approaches. Functional annotation was performed mainly to yield insights into the enzymes involved in epigenetic modifications, which could be relevant to its development. By using Hidden Markov Models, we have identified a total of 54 and 39 HME proteins in the predicted proteomes of *S. mansoni* and *S. japonicum*, respectively. The results show that *S. mansoni* and *S. japonicum* code for proteins in all the selected HMEs families, with the largest number of proteins found in the HMT subfamilies. Individual annotations of *S. mansoni* proteins revealed 14 splicing variants as well as some incorrect predictions identified in both species. Only six HMEs had been experimentally studied and the others were previously annotated only by automatic sequence similarity-based methods as described elsewhere. Thus, we have improved the annotation of 23% of *S. mansoni* HMEs. As we continue this work, we will validate some HMEs as molecular targets using RNA interference to silence the corresponding genes in schistosomula and analyze the resulting knockdown by quantitative PCR and potential phenotypes.

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SCHISTOSOMA MANSONI TEGUMENT (SMTEG) MODULATES THE EXPERIMENTAL ALLERGIC ASTHMA

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Allergic inflammations are directed by Th2 cells activation that produces large amounts of IL-4, IL-5 and IL-13. These inflammatory mediators induce IgE production and eosinophilia. The schistosomula is the first stage to keep contact with the host immune system activating the antigen presenting cells and promoting the lymphocytes B and T differentiation. Although immune responses between helminthes infections and asthma are similar some studies performed in endemic areas of *Schistosoma mansoni* showed low prevalence of allergic diseases. This modulation has been associated to interleukin (IL)-10 production and an increased number of T regulatory cells. Many factors may be acting to inhibit the allergic asthma induction in mice associated to schistosomiasis, which involve both innate immune responses as adaptive. Our goal is to investigate the mechanisms responsible for the immune modulation of asthmatic response after the Smteg (*S. mansoni* tegument preparation) intraperitoneal injection. Balb/C mice were divided in three groups (n=5) PBS, Asthma and Smteg/Asthma. All groups were immunized twice with 10µg of ovalbumin chicken egg (OVA) plus alum subcutaneously in a 15 days interval. One week after the first one, mice from Smteg/Asthma group were immunized intraperitoneally with 25 µg of Smteg. The ASTHMA groups were challenged by OVA aerosol to develop asthma, one week after the second immunization and after twenty four hours mice were euthanized. Bronco-alveolar lavage (BAL) was performed to eosinophils counting and the lungs were collected for cytokines (IFNγ, IL-4, IL-10, IL-17, IL-13) and chemokines (CCL2, CCL3, CCL5, CCL11) analysis. The number of eosinophils was higher in ASTHMA group compared to PBS. SMTEG/ASTHMA presented reduced levels in CCL11, IL-17, IL-13 and eosinophils numbers and high levels in interleukin 10 (IL-10) when compared to ASTHMA group. IL-4, CCL2 and CCL3 did not show statistical difference between these groups. Treatment with Smteg modulated the number of eosinophils with increase in IL-10 in allergic asthma.

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VACCINE EFFICACY OF THE *SCHISTOSOMA JAPONICUM* INSULIN RECEPTORS

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Schistosomiasis, which affects 200 million people worldwide and is responsible for hundreds of thousands of deaths annually, continues to be a significant public health problem. We have identified two types of insulin receptors from the blood fluke, *Schistosoma japonicum*, SjIR-1 and SjIR-2. SjIR-1 is located on the tegument basal membrane and the internal epithelium of adult worms, whereas SjIR-2 is located in the parenchyma of males and the vitelline tissue of females. The incubation of adult worms *in vitro* with HNMPA, which inhibits autophosphorylation of the HIR that is involved in the regulation of glucose uptake in mammalian cells, and anti-SjIRs antibodies respectively resulted in a significant decrease in worm glucose and glycogen levels, suggesting the important role of SjIRs in regulating glucose uptake, similarly to that described for mammalian cells. Vaccination of mice with recombinant SjIRs followed by cercarial challenge infection with *S. japonicum* resulted in statistically significant the stunting of adult worms ranging from 22-25% in the SjIR-1 vaccinated group to 37-42% in the SjIR-2 vaccinated groups, highly significant reductions in faecal eggs in both the SjIR-1 (66%) and SjIR-2 (68%) vaccinated groups, although there was no significant reduction

in adult worm burdens. Vaccination also resulted in a reduction in liver egg numbers in the SjIR-1 (33%) and SjIR-2 (5.4%) vaccinated groups. Based on repeated vaccination trials, the resulting reductions in worm length, liver and intestine egg numbers and faecal eggs all indicate a significant depression of parasite growth and a subsequent reduction in parasite fecundity. The highly significant decreases in faecal egg output is noteworthy and suggests an application as a veterinary vaccine which could prevent transmission of zoonotic schistosomiasis to the human endemic population. Further development and validation of the vaccine is currently underway. This work also provides important new information on the role of SjIRs in the biology of *S. japonicum*, and may suggest their novel use as vaccine targets against schistosomiasis and other debilitating parasitic diseases.

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TEMPO-SPATIAL DISTRIBUTION OF *SCHISTOSOMA JAPONICUM* IN THE YANGTZE RIVER

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Schistosomiasis is caused by contact with schistosome-infested water when washing or bathing. The flooding period between May and September each year of the Yangtze River, is the high-risk season of infection of *Schistosoma japonicum*, and the resultant high re-infections. The present study was to investigate the tempo-spatial distribution pattern of *S. japonicum* cercariae in waterbody of Jiangsu section of the Yangtze River. The water infectivity of *S. japonicum* was determined along the the Yangtze River from May to September by using sentinel mice, and the dynamic database of water infectivity was established. Among the 4 500 sentinel mice which were placed in 45 sites, 4 411 were recovered, with a recovery rate of 98.33%. A total of 4 370 mice were dissected and 23 infected, with a total infection rate of 0.53%, and the infection rates of *S. japonicum* in mice were 0.23%, 0.23%, 0, 0.45% and 1.73%, respectively month by month. Fifty-five adult worms were collected, with mean worm burden of 2.39 worms per mouse in infected sentinel mice, and the mean worm burdens of the infected sentinel mice were 1.00, 2.00, 0, 1.50 and 2.87 worms per mouse, respectively month by month. From May to September, 12 sites with infected sentinel mice were found, accounting for 24.44% of the total forecast and surveillance sites, and number of sites with infected sentinel mice were 1, 2, 0, 1 and 8, respectively, with occurrence rates of sites with infected mice of 2.22%, 4.44%, 0, 2.22% and 17.78%, respectively, and the constituent ratios of the sites with infected mice were 8.33%, 16.67%, 0, 8.33% and 66.67%, respectively month by month. The occurrence rate of sites with infected mice in September was significantly higher than that in June (χ^2=4.05, *P*=0.044). And the top infection rates of *S. japonicum* in sentinel mice were found in sluices, being 2.08%-6.45%. It is concluded that the infection of *S. japonicum* during the period of flood season in the Yangtze River exhibits bimodal distribution. Top water infectivity appears in September, July is the period of metagenesis of infected snails in marshland areas, and the critical time for prevention and control of acute schistosomiasis is between August and October.

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PREVALENCE OF URINARY SCHISTOSOMIASIS IN OGUN STATE, SOUTHWEST NIGERIA

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This study, initiated by the Federal Ministry of Health Abuja, Nigeria, is aimed at providing prevalence distribution data of urinary schistosomiasis in Ogun State, Nigeria, as part of an ongoing effort in planning a national control program of the disease by preventive chemotherapy. In October 2009, a cross-sectional prevalence survey of *Schistosoma haematobium* infection among school children aged 9-11 years was carried out in 15 of the 20 local government areas (LGA) of Ogun State, Nigeria. One study community was selected in each LGA. Following informed consent from the parents and community leaders, urine samples were collected in clean specimen bottles from 50 randomly selected pupils from each of the 15 study communities. The geographical coordinates of the study communities were recorded. The urine samples were examined visually for macrohaematuria, tested with reagent strips for microhaematuria and examined microscopically for *S. heamatobium* eggs. Positive diagnosis was based on detection of macrohaematuria, microheamaturia and / or *S. haematobium* eggs in urine. Out of 735 pupils (367 males and 368 females) examined, 194 (26.4%) were positive for *S. heamatobium* infection. The infected children were found in 13 of the 15 LGAs visited. The 194 infected, represent a prevalence of 30.4% of the 638 children examined in the 13 endemic LGAs. Prevalence in the endemic communities varied from the lowest of 2% at Idode in Ijebu North LGA to the highest of 84% at Imala Odo in Abeokuta North LGA. Prevalence was above 20% in seven LGAs, that is, Odeda, Abeokuta North, Yewa South, Yewa North, Obafemi Owode, Ewekoro and Ijebu East. Prevalence was highest (≥ 40%)among the fishermen communities situated on the banks of or close to water reservoirs in water development project areas of the state. Prevalences of infection among the two sexes were comparable with 27.8 % in the males and 25.0% in the females (p>0.05)

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CHARACTERIZATION OF THE NOVEL SCHISTOSOMAL RECEPTOR, SMGPR-3

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The main causative agent of schistosomiasis, a disease which infects over 200 million people worldwide, is the parasite *Schistosoma mansoni*. Treatment of the disease is primarily with praziquantel (PZQ). There is an increasing fear that with the widespread use of the drug, and the lack of an available alternative, PZQ will lose its effectiveness. We are currently researching the schistosome nervous system to gain insight into this area. The nervous system coordinates many vital functions in the worm and is considered to be an excellent target for anti-schistosomal drugs. Recently, we have discovered a new group of schistosomal biogenic amine (BA) G-Protein Coupled Receptors (GPCRs), the smGPRs, which likely have a neuronal function. SmGPR-1, -2 and -3 have been cloned, and their pharmacological profiles determined by our lab. These receptors differ from the BA GPCRs of the human host in both sequence and function. SmGPR-3 was immunolocalized in the adult worm and shown to be expressed abundantly in the central and peripheral nervous system, including peripheral neurons which innervate the worm musculature, indicating a neuromuscular role. To further characterize smGPR-3, a predicted binding site (D3.32) was mutated and functional expression studies were performed, to assess receptor activity. The wild-type and mutant plasmid constructs were individually expressed in yeast cells which, upon successful interaction with ligand and receptor activation, express a reporter gene, allowing for the cells to be selectively grown in media. Cell growth is then quantitatively assayed, using the Alamar Blue fluorescence assay, as a measure of receptor activation. The mutants showed varied levels of responsiveness to ligand, indicating the site's importance. RNAi knock-down of smGPR-1, -2, and -3 in schistosomulae, followed by video analysis was performed. Targeting of smGPR-3 results in a decrease in worm motility by approximately half, as compared to the control, indicating a role in motility for the receptor.

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SUPPRESSION OF IMMUNOPATHOLOGY IN MURINE *SCHISTOSOMIASIS MANSONI* BY A TARGETED PLASMID CONTAINING IMMUNOTOXIN, DT_{390}-IL-18-SRA GENE (STUDIES OF *IN VITRO* AND *IN VIVO* EFFICACY)

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This study was undertaken to evaluate both *in vitro* and *in vivo* effects of DT_{390}-IL-18-SRα immunotoxoin on the development of immunopathology in murine schistosomiasis mansoni. The antiproliferative effect and *in vitro* granuloma formation (IVGF) inhibition were evaluated using methyl thaiazolyl tetrazolium and IVGF index respectively. *In vivo* evaluation, mice were divided into four groups of twelve mice each: Group1 normal non infected (-ve control), Group 2, 3 and 4 were infected percutaneously with 25 cercariae. After 6 weeks post infection, Group2 was treated with PBS (+ve control), Group3 was treated with plasmid DNA (50 µg) embedded with cationic liposome in 50 µl PBS, injected intramuscular in the hind limbs once daily for two weeks and Group4 was treated with DT_{390}-SRα (mutant control). After animals sacrification, liver was removed for histopathological studies. DT_{390}-IL-18-SRα showed suppression of spleen lymphocytes proliferation and IVGF in a dose dependant manner as well as suppression of liver granulomas size by 80%, while mutant toxin had no significant suppression (P › 0.05). In conclusion, the immunotoxin showed selective toxicity to antigen activated lymphocytes *in vitro* with reduced clinical and pathological severities of the disease.

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ACETYLCHOLINE-GATED CHLORIDE CHANNEL SUBUNITS AS MODULATORS OF *SCHISTOSOMA MANSONI* MOTOR FUNCTION

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Currently, praziquantel is the only treatment available against parasitic trematodes of the genus *Schistosoma*. Thus, discovery of new drug targets against schistosomes is of the utmost importance. Historically, drugs targeting modulators of worm motility, particularly the cholinergic system, have been effective against helminth parasites. Previous studies have shown that acetylcholine and other cholinergic agonists have strong paralytic effects on schistosomes *in vitro*. Paralysis is mediated by nicotinic acetylcholine receptors (nAChRs) and is associated with relaxation of body wall muscles, suggesting an inhibitory neuromuscular effect. Analysis of the *S. mansoni* genome database has revealed that in addition to the expected, excitatory cation-selective nAChRs, there are several nAChRs predicted to form putative anion-selective channels. As cation channels are not known to cause muscle relaxation, we hypothesize that the putative anion nAChRs are responsible for the inhibitory effects of acetylcholine on schistosome movement and are promising drug targets due to their low homology to other nAChRs. The goal of this study is to characterize the putative anion-selective nAChR subunits of *S. mansoni*. Here, we present the results of a bioinformatics analysis of schistosome cholinergic receptors and an RNAi study assessing the role of putative anion-selective nAChRs in *S. mansoni* motor function. SiRNA against 5 nAChR subunits were transfected into Day 0 schistosomulae. Motor phenotypes were then quantified using motion-tracking software both at baseline levels and after treatment with exogenous acetylcholine. We observed significant increases in the frequency of body wall contractions and overall body length in the siRNA-treated samples compared to the negative control. Treatment with exogenous ACh caused no change in phenotype of the siRNA-treated samples but did lead to increases in length in the controls. These results suggest that ACh may act through these putative anion-selective nAChRs as an inhibitory neurotransmitter affecting worm motility.

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EXPRESSION AND ANALYSIS OF *SCHISTOSOMA MANSONI* IMMUNODIAGNOSTIC ANTIGEN, SM29

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The helminthic parasite *Schistosoma mansoni* is one of the causative agents of the neglected tropical disease schistosomiasis, which affects more than 200 million people worldwide. Since 1985, several of the more reliable methods for immunodiagnosis of this disease, FAST ELISA and EITB, have relied on a mixture of proteins from the microsomal fraction of adult worms. The *S. mansoni* microsomal protein preparation contains two species-specific antigens known as Sm29 and Sm25, based on their migration by gel electrophoresis. These proteins have demonstrated excellent sensitivity and specificity by Western blot; however, they have proven to be both expensive and difficult to obtain. The release of the *S. mansoni* genome provides an opportunity to utilize a proteomics approach for the identification of these diagnostic markers and the potential to develop a less expensive, more abundant source of antigen as recombinant protein. For identification of Sm29 the microsomal antigen fraction was analyzed by two-dimensional gel electrophoresis revealing 15 distinct protein spots at the correct molecular weight, 5 of which were immunoreactive by Western blot analysis. Spots corresponding to the Western blot positive proteins were excised from the gel, digested with trypsin and analyzed by mass spectrometry, which revealed each protein came from the same gene product. The gene has been cloned and recombinant Sm29 protein was expressed in a baculovirus expression system, and subsequently tested as an immunodiagnostic marker for *S. mansoni* infection.

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SMTOR IS A NEW CANDIDATE VACCINE FOR SCHISTOSOMIASIS

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Praziquantel is the only chemotherapeutic available for treatment of infection with *Schistosoma* spp., but it does not protect from re-infection. Therefore, there is a need to develop an effective vaccine against schistosomiasis. Vaccine candidates include proteins located on the parasite tegument such as SmTSP-2, Sm29 and SmStoLP-2 and the cytosolic fatty acid binding protein Sm14, but only the last-mentioned is in the clinical trial phase. Here we explored another surface-exposed vaccine target. SmTOR is a tetraspanning orphan receptor expressed highly in *S. mansoni* cercariae. Its extracellular domain 1 (ed1) contains a complement C2 binding sequence that had been described for the *S. haematobium* receptor homologue (ShTOR) and been shown to interfere with complement C2 cleavage *in vitro*. We recombinantly over-expressed SmTORed1 in E. coli, tested its capacity to bind purified C2 and furthermore the occurrence of specific anti-rSmTORed1 antibodies in *S. mansoni* infected and uninfected individuals. To ensure antibody specificity, we performed a competitive ELISA by pre-incubating the positive sera with recombinant SmTORed1 produced as HaloTag fusion protein coupled to a solid support. Lastly, rSmTORed1 was tested as vaccine candidate in a murine infection and challenge model. Purified rSmTORed1 bound complement C2 alike the peptide motif found in ShTOR. We detected specific antibodies against rSmTORed1 in 2/20 (10 %) patient sera and 2/40 (5 %) sera of uninfected individuals. The low occurrence of antibodies in patient sera is possibly due to SmTOR is not being recognized during infection. That uninfected individuals have such antibodies might be due to infection with bird schistosomes (Trichobilharzia). Balb/c mice immunised with rSmTORed1 with CFA/IFA generated significantly high antibody titres that were protective against infection as shown by a significant decrease in worm burden in immunised

versus control animals (60 % reduction). In conclusion, we found that rSmTORed1 is a promising new vaccine target against *S. mansoni* infection.

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A VASA GENE FROM *SCHISTOSOMA MANSONI* FOR DEVELOPMENT AS A MARKER FOR GERMLINE TRANSGENESIS

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Although retrovirus mediated somatic transgenesis in *Schistosoma mansoni* has been reported previously, an important goal is the development of germline transgenesis. Progress towards this might be monitored if germ cells could be identified in cultured schistosomes. Vasa, an ATP-dependent DEAD-box RNA helicase, has germline-specific expression. A reporter transgene driven by a schistosome vasa-like promoter might reveal whether transgenes had reached the germ cells. The present study addressed the identification of a vasa-like gene in *S. mansoni* and characterization of putative promoters of schistosome vasa to drive expression of the firefly luciferase reporter gene. Our findings indicate that the *S. mansoni* genome encodes three putative vasa orthologues identified by BLAST searches and related bioinformatics tools. Phylogenetic relationships were inferred using PHYLIP. The vasa-like orthologues were termed *S. mansoni* vasa-like gene 1 (Smvlg1), Smvlg2, and Smvlg3. A 1.5 kb and 2.0 kb genomic fragment of the Smvlg1 promoter was cloned into pGL3 and was employed to transfect the HT1080 human fibrosarcoma and the HeLa cervical cancer cell lines. Lysates of transfected cells were analyzed for luciferase activity. Also, schistosome eggs were transformed with the Smvlg1 promoter constructs. Total RNA isolated from miracidia hatched from transfected eggs was retrotranscribed into cDNA and luciferase reporter transgene expression quantified by real time PCR. These findings indicated that the 1.5 kb and the 2.0 kb promoter fragments of Smvlg1 were capable of driving reporter gene expression in mammalian cancer cell lines and that the 2.0 kb promoter fragment capable of driving reporter gene expression more efficiently than the 1.5 kb fragment in *S. mansoni* eggs.

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LENTIVIRAL TRANSDUCTION OF THE HUMAN BLOOD FLUKE *SCHISTOSOMA MANSONI*

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We have begun to address whether pseudotyped human immunodeficiency virus type 1 (HIV-1) (a lentivirus) might have utility for transgenesis and other functional genomics in schistosomes. We investigated early steps of lentivirus infection including attachment of virions to the schistosome tegument, reverse transcription to synthesize viral cDNA, and integration of the provirus into the schistosome genome. 293T/17 producer cells were transfected with plasmid encoding wild type HIV-1 isolate NL4-3 and vesicular stomatitis virus-glycoprotein (VSV-G) encoding plasmid to produce lentivirus virions pseudotyped with VSV-G. Schistosomes were incubated with VSVG-HIV virions in the presence of polybrene. At intervals from 0 to 4 hours, schistosomes were washed and the surface proteins cross-linked with formalin. Using a VSV-G specific antibody, time course dependent immunolocalization of VSV-G was observed to both schistosomules and adult worms, with fluorescence signals increasing with time. Downstream events were investigated at days one and two after transduction. Total DNA was extracted from schistosomes and used as template for quantitative real-time PCR measuring products of reverse transcription and integration. One step PCR for reverse transcription products revealed the presence of strong stop and positive strand viral cDNA, and anchored PCR indicated the integration of HIV proviruses in schistosome genome. Findings with control groups exposed to heat inactivated virions and with spinoculation of virions onto the schistosomes provided additional support to the hypothesis that proviral lentiviral transgenes had integrated into schistosome chromosomes. Future studies will address chromosome/ provirus integration junctions, target site preferences and reporter gene activity, and with the aim of establishing VSV-G-pseudotyped HIV-1 as a vector for genetic manipulation of schistosomes.

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SEVERE *SCHISTOSOMIASIS MEKONGI* IN SOUTHERN LAO PEOPLE'S DEMOCRATIC REPUBLIC

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In 2007, within the context of a community-based survey on helminth infections in three villages in Khong District of southern Lao People's Democratic Republic, we identified severe cases of schistosomiasis. We revisited three villages yearly and followed the patients for three years. We identified nine patients with severe schistosomiasis (7 male, 2 female). Mean age of the nine patients was 36 years (range: 5 - 66 years). The leading clinical features were cachexia, hepatosplenomegaly, ascites, splenic varices and rupture of oesophageal varices. Patients were co-infected with *Opisthorchis viverrini* (*n*=6), *Strongyloides stercoralis* (*n*=1) and hookworm (*n*=7). All patients were treated with praziquantel. Three patients improved (case 5, 6, 9), two adult patients (case 2, 3) remained unchanged or the status worsened. Two patients (case 4, 7) died due to oesophageal bleeding. Two new patients were diagnosed in 2009 (case 7, 8). Liver pathology improved after treatment in particular in young patients. Severe chronic schistosomiasis is still present in Laos. Schistosomiasis transmission is currently ongoing as documented by the presence of diseased children. A long-term integrated control intervention including access to treatment, health education, sanitation and infrastructure are urgently required.

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DIHYDROARTEMISININ, A NEW ANTISCHISTOSOMAL AGENT AGAINST *SCHISTOSOMA JAPONICUM*

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Praziquantel is virtually the only current drug of choice for treatment of human schistosomiases. However with the extensive, long-term repeated use of the drug for morbidity control, there is a growing concern that praziquantel resistance or reduced susceptibility may emerge. Screening and development of novel antischistosomal agents, is therefore given high priority. It has been shown that artemisinin derivatives like artemether and artesunate exhibit effectively antischistosomal activities. However, the antischistosomal efficacy of dihydroartemisinin, the main metabolite of the mother compound artemisinins, as well as of the two derivatives, artemether and artesunate, remains unclear. The present study was designed to investigate the *in vivo* activity of dihydroartemisinin against S. *japonicum*. Our finding showed that, single oral doses of dihydroartemisinin (at 300 mg/kg) reduced total worm burdens of 1.07%-64.81% and female worm burdens of 11.90%-90.48%, depending on when, relative to infection, treatment was given, and the greatest reductions was seen when treatment was given either 7 or 35 days post-

infection. However, no marked dose-response relationship was observed. During the schistosomulum stage (7 day), the combined treatment of dihydroartemisinin and praziquantel, or administration of praziquantel, followed by treatment of dihydroartemisinin, both resulted in lower efficacies of dihydroartemisinin against *Schistosoma japonicum*. However, no marked changes of antischistosomal activities were observed when dihydroartemisinin was given first, followed by praziquantel. At adult stage (35 day), a significantly higher antischistosomal efficacy was found for combination therapy with dihydroartemisinin given first, followed by praziquantel, compared to dihydroartemisinin alone, or praziquantel given first followed by dihydroartemisinin. However, no significant difference was observed between the effects of combined treatment of dihydroartemisinin and praziquantel and administration of praziquantel alone. Administration with artemether, artesunate and dihydroartemisinin at multiple doses or in combined treatment damages both juvenile and adult *S. japonicum*, but there were no statistically significant differences among the three drugs at the same dose. It is concluded that, dihyartemisinin is a novel antischistosomal agent against *S. japonicum*.

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TRANSGENE EXPRESSION FROM *MOS-1 MARINER* TRANSPOSON IN *SCHISTOSOMA MANSONI*

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The genome sequence of the *Schistosoma mansoni,* one of the major causative agents of schistosomiasis worldwide, is available in draft format. This genome includes about 12,000 protein encoding genes. The function of most of these remains unknown or poorly understood but, can represent new targets for intervention and control. Insertional mutagenesis of exogenous transposons has been shown to be powerful transgenesis tools as in many species. Previously we have reported that the *piggyBac* transposon is active in schistosome tissues and can integrate into the schistosome genome. The *Mos-1 mariner* transposon, originally isolated from the fruit fly belongs to one of the most widespread transposable element family. As with *piggyBac*, we have been investigating the potential utility of this transposon for functional genomics in schistosomes. Using an excision assay that analyses the donor plasmid backbone, we have also recently determined that *Mos-1 mariner* is transpositionally active in schistosome tissues. In the present study, we have focused on the activity and longevity of the reporter transgene, firefly luciferase, carried as cargo within the inverted terminal repeats of the transposon, using RT-PCR based approaches. Targeting the schistosomule stage of *S. mansoni* at the outset, we investigated the effect of age of the schistosomules on luciferase expression after introduction of the transposon. Second, we investigated the influence of chromosomal integration of the transposon by comparing expression in schistosomules that had been transduced with the donor transposon alone compared with others transduced with the donor plasmid transposon in tandem with mRNA encoding the *Mos 1 mariner* transposase. Third, we monitored the time course of expression and longevity of expression of the luciferase after exposure of the schistosomules to *Mos-1 mariner.* These findings and discussion of the impact of the results will be discussed.

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APPLICATION OF MICROSATELLITE GENOTYPING TO CERCARIAE IN THE INVESTIGATION OF URBAN SCHISTOSOMIASIS

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Schistosomiasis is an endemic disease to parts of Brazil that affects 2-6 million people in 9 states. Caused by *Schistosoma mansoni*, whose intermediate hosts are snails of the species *Biomphalaria glabrata*, being the only transmitter found in the state of Bahia. Historically described as a rural disease, urban transmission of the parasite has been more commonly seen in cities of Brazil. Our goal was to determine the utility of cercariae shed from collected snails in estimating the genetic relationships between urban populations of parasites. Taking advantage of an ongoing malacologic study of all major collections of water in the city of Salvador (total of 158) seven sites were identified as positive for cercariae shedding. Cercarial DNA from 5 sites was extracted and quantified by qPCR. Genotyping assays were performed using 14 microsatellite markers and diversity index used was the Jost's D. Worm and cercariae DNA from laboratory strain maintained at Case Western Reserve University and at Oswaldo Cruz Foundation, respectively, were used as PCR positive control. The total number of alleles observed for all of the markers was 120, ranging from 44 to 91, in the district of Itacaranhas and Pituaçu, respectively. The average effective allele number was similar across all samples. A pairwise comparison of the Jost's D values of all cercarial collections showed a great amount of differentiation and difference between most field collections was as great as that between the positive controls. Only two collections demonstrated potential gene flow between them, the laboratory strain Feira de Santana and Dique do Cabrito (mean Jost D = 0.017). This, however, is spurious since they are reproductively isolated from each other. Therefore, there was no correlation between geographic location and diversity indices. Our results suggest some evidence that snail infections may not reflect the genetic diversity found in the associated human population and that in Salvador, snail examinations may not be useful to assess parasite population structure and dynamics in the human host.

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SPATIAL AND TEMPORAL STABILITY OF *GLOSSINA FUSCIPES FUSCIPES* POPULATIONS IN UGANDA

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Glossina fuscipes, a riverine species of tsetse, is the major vector of human African trypanosomiasis (HAT) in sub-Saharan Africa. Understanding the population dynamics, and specifically the spatial and temporal stability, of *G. fuscipes* will be important for informing vector control activities. We evaluated spatial and genetic changes over time in twelve populations of the subspecies *G. f. fuscipes* distributed across southeastern Uganda, including a zone of contact between two historically isolated lineages. A total of 861 tsetse flies were genotyped at 16 microsatellite loci and at one mitochondrial locus. Results of an AMOVA indicated that time of sampling did not explain a significant proportion of the variance in allele frequencies observed across all samples. Estimates of differentiation between samples from a single population ranged from approximately 0 to 0.019, using Jost's DEST. Effective population size estimates using

momentum-based and likelihood methods were generally large. We observed significant change in mitochondrial haplotype frequencies in just one population, located along the zone of contact. The change in haplotypes was not accompanied by changes in microsatellite frequencies, raising the possibility of asymmetric mating compatibility in this zone. In conclusion, our results suggest that populations of *G. f. fuscipes* are large and stable over the 8-12 generations studied. Karuma and Kafu are additional areas of overlap of the southern and northern flies' populations. Future studies should aim to reconcile these data with observed seasonal fluctuations in the apparent density of tsetse.

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SANDFLY SURVEILLANCE AND THE DEVELOPMENT OF A LEISHMANIASIS RISK ASSESSMENT IN EAST AFRICA

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The leishmaniases represent a group of neglected tropical diseases found throughout the Horn of Africa (HOA) and East Africa. The current prevalence is largely unknown and underreported. Phlebotomus sand flies are the vectors in the old world. *Phlebotomus orientalis* is the proven vector for visceral leishmaniasis (VL) in Sudan and Ethiopia while *P. martini* is the vector in Kenya and Uganda. Accurate information on sand fly population density, distribution, and species diversity is crucial for the development and implementation of targeted prevention and control efforts. The objective of this study was to assess vector diversity, distribution and relate this to endemic leishmania infection rates in sand flies using GIS mapping techniques. The end state is to develop a surveillance system for detecting and monitoring changes in these variables in East Africa. Sampling was done in five sites in Kenya (Isiolo, Garisa, Wajir, Lamu and West Pokot), and two in Tanzania (Arusha and Kilimanjaro regions).Sand flies were collected using CDC light traps baited with 0.5 kg dry ice from October 2008 to April 2010. Sites in Kenya were visited twice per year while Tanzania once in July 2010 for a period of five nights on each visit. *Leishmania* infections were identified using standard genetic analysis. A conventional Polymerase Chain Reaction (PCR) assay confirmed presence/absence of the parasite and a real-time PCR assay was subsequently run for speciation. Over 20,000 sand flies were collected. A representative sample of 6,843 specimens was identified. *P. orientalis* was found in Isiolo (974), Wajir (328) and Garissa (620) while *P. martini* in Garissa (2) West pokot (78) and Tanzania (17). Sergentomyia species were found in all sites. PCR results are ongoing and will be presented in another forum. The presence of *P. orientalis and P. martini* in Garissa and *P. martini* in Tanzania are the first to be documented. This suggests that there is increasing potential for VL outbreaks in areas of East Africa considered to be low risk.

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NIGERIA *ANOPHELES* VECTOR DATABASE: A REVIEW OF 100 YEARS' RESEARCH

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Anopheles mosquitoes are important vectors of malaria and lymphatic filariasis (LF) which are major public health diseases in Nigeria. Malaria is caused by infection with a protozoan parasite of the genus *Plasmodium* and LF by the parasitic worm *Wuchereria bancrofti*. Knowledge of the *Anopheles* vectors that transmit these diseases is necessary in order to plan vector control appropriately in Nigeria. To present a comprehensive report on the spatial distribution and composition of these vectors, all published data available were collated into a database. Details recorded for each source were the locality, latitude, longitude, time/period of study, species, abundance, sampling and collection methods, morphological and molecular species identification methods, insecticide resistance status, including evidence of the KDR allele, and *Plasmodium falciparum* sporozoite rate and *W. bancrofti* microfilaria prevalence. This collation resulted in a total of 107 publications, encompassing 481,661 vector *Anopheles* mosquitoes in 628 spatially unique descriptions at 145 geo-referenced locations being identified across Nigeria from 1912 to 2010. Overall, the highest number of vector species reported included *An. gambiae* complex (65.6%), *An. funestus* complex (17.5%), *An. gambiae* s.s. (6.4%). *An. arabiensis* (5.0%) and *An. funestus* s.s. (2.3%), with the molecular forms *An. gambiae* M and S identified at 120 locations. A variety of sampling, collection and species identification methods were used over time with an increase in molecular techniques in recent decades. Insecticide resistance to pyrethroids and organochlorines was found in the main *Anopheles* species across 45 locations. Presence of *P. falciparum* and *W. bancrofti* varied between species with the highest sporozoite rates found in *An. gambiae* s.s, *An. funestus* s.s. and *An. moucheti*, and the highest microfilaria prevalence in *An. gambiae* s.l., *An. arabiensis*, and *An. gambiae* s.s. This comprehensive geo-referenced database provides an essential baseline on *Anopheles* vectors and will be an important resource for malaria and LF vector control and elimination programmes in Nigeria.

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HABITAT ASSOCIATIONS OF EASTERN EQUINE ENCEPHALITIS IN THE FLORIDA PANHANDLE

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Eastern Equine Encephalitis virus (EEEV) is an alphavirus with high pathogenicity in both humans and horses. In 2010, Florida had four human fatalities of EEEV and Florida continues to have the highest amount of human cases in the USA. Furthermore, Florida sees year-round EEEV transmission, whereas other states have a more pronounced seasonal pattern. Florida's habitat is uniquely different from the other 48 continental states in that it has both tropical and sub-tropical regions. There are higher levels of EEEV transmission in the panhandle and northern regions of Florida as compared to the central and southern areas. To determine which habitats play a role in EEEV transmission in the Florida panhandle, 24 sentinel sites were categorized as enzootic, periodic, and negative based on the number of chicken seroconversions to EEEV from 2005-2009. The average EEEV prevalence rate in the sentinel chickens across all sites over the last five years was 3.4%, with the most active site's sentinel flock averaging a prevalence rate of 11.95%. A habitat analysis was conducted using Arc GIS 9.3 on all 24 sites, using level two land cover classifications. The land classification data was analyzed using an analysis of variance and comparisons were made between enzootic, periodically enzootic, and negative sites. The analysis of variance produced results showing both risk and protective ecological factors for EEEV transmission. The ecological risk factor found to be associated with higher levels of EEEV transmission was the tree plantation habitat. Protective ecological factors associated with reduced levels of EEEV transmission were vegetated non-forest wetland and wetland coniferous forest habitats. In identifying tree plantations as a habitat of risk, surveillance programs can target these areas for monitoring and treatment, thereby potentially reducing the risk of EEEV in both the human and horse populations within Florida.

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CHARACTERIZATION OF PERITROPHINS FROM THE SAND FLY *PHLEBOTOMUS PAPATASI*

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The sand fly peritrophic matrix (PM) plays a key role in compartmentalization of the blood meal and as barrier to *Leishmania*. However, little is known about sand fly PM molecular components and structural organization. We characterized three peritrophins (PpPer1, PpPer2, and PpPer3) from Phlebotomus papatasi. PpPer1 and PpPer2 display, respectively, four and one chitin-binding domains (CBDs). PpPer3 on the other hand has two CBDs, one mucin-like domain, and a putative domain with hallmarks of a CBD, but with changes in key amino acids. Temporal and spatial expression analyses show that PpPer1 is expressed specifically in the female midgut after blood feeding. PpPer2 and PpPer3 mRNAs were constitutively expressed in midgut and hindgut, with PpPer3 also being expressed in Malpighian tubules. PpPer2 was the only gene expressed in developmental stages. Recombinant PpPer1, PpPer2 and PpPer3 were obtained and shown to display similar biochemical profiles as the native proteins. Our data indicate that rPpPer1 and rPpPer2 are able to bind chitin, suggesting they are involved in PM formation, and likely are also involved in heme detoxification based on their ability to bind heme. In contrast, the mucin-like PpPer3 appears to be involved in protecting the midgut epithelia, and is only expressed in the pyloric triangle. PpPer1 and PpPer3 expression are regulated by Le. major infection. Interestingly, knock down of PpPer1 led to 45% reduction in mRNA levels and 44% in protein which resulted in increases of parasite load of 39% at 48h and 22% at 96h post-infection. The results support the role of PpPer1 as a component of the PM scaffold and may strongly suggest that PpPer1 significantly contributes to the PM overall structure organization and porosity. PpPer1 appears to be a key determinant of the PM role as a barrier to *Leishmania*.

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BURROWING SCABIES MITES ALTER SKIN CELL GENE EXPRESSION

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We have previously demonstrated that the ectoparasitic mite, *Sarcoptes scabiei,* is the source of substances that modulate the cytokine secretion and adhesion molecule expression of host skin cells including epidermal keratinocytes, dermal fibroblasts and microvascular endothelial cells. We have also shown that live mites burrowing into the surface of a human skin equivalent (keratinocytes and fibroblasts in a collagen matrix; HSE) induce the secretion of a variety of both pro- and anti-inflammatory cytokines. Among the most significantly induced cytokines were interleukin-1α (IL-1α), IL-1β, IL-1 receptor antagonist (IL-1ra), IL-6, IL-8, T cell-attracting chemokine (CTACK), monocyte chemoattractant protein-1 (MCP-1), and macrophage- and granulocyte/macrophage colony-stimulating factors (M-CSF and GM-CSF). In this study, we sought to determine if mite burrowing was also able to elicit parallel changes in gene expression by these skin cells. Live scabies mites were allowed to burrow into HSEs for 48 hrs then tissues were frozen at -80°C in RNA*later.* RNA was extracted and subjected to gene expression profiling using Affymetrix GeneChip® Human Gene 1.0 ST microarrays at the Wright State University Center for Genomics Research. Genes for several cytokines were among the most up-regulated by live mites. The gene ontology groups comprised of genes involved in IL-1/IL-1ra activity, tumor necrosis factor production (TNF), IL-6, and vascular endothelial growth factor (VEGF) regulation and production represented 4 of the top 6 groups modulated by live scabies mites. These data indicate that the cytokine secretion induced by live mites burrowing into HSEs is regulated at the gene expression level.

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EVALUATION OF ULTRA LOW VOLUME AND THERMAL FOG PESTICIDE APPLICATIONS AGAINST OLD WORLD PHLEBOTOMINE SAND FLY VECTORS OF *LEISHMANIA* IN KENYA

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One component of the Department of Defense (DoD) pest management system is ultra-low volume (ULV) and/or thermal fog aerosol pesticide application. Despite widespread implementations of this and other components of the system, such as use of repellents and permethrin, US military operations in hot-arid regions still face substantial impacts from insect vectors of disease such as mosquitoes and sand flies. Few studies have compared ULV and thermal fog technologies, and no study has analyzed their performance or efficacy against sand flies in hot-arid environments. In this study we evaluated the Grizzly ULV (Clarke) and the Swingfog SN101E (Swingtec) calibrated on site with two pesticides, Fyfanon (malathion) and Duet (sumithrin, prallethrin, and PBO), in separate trials against caged sentinel *Phlebotomus duboscqi* sand flies and wild populations of *Phlebotomus* and *Sergentomyia* spp. sand flies in the hot-arid North Rift Valley, Kenya. Wild sand fly populations were sampled throughout the study and for all trials sentinel sand flies were arranged in 25-cage grids with five offsite control cages. Spray plots for both the sprayers and chemicals were reciprocated and spray times and environmental conditions were reasonably consistent across trials. Wild sand fly population sampling showed good control in all treated plots as well as a possible repellent effect indicated by increased populations in nearby untreated areas. Wind shear effect was observed in spatial mortality patterns in thermal fog applications, but was notably absent in mortality from concurrent ULV applications. Prior trials with the Grizzly in Kenya demonstrated widespread control with Duet, but the reverse was seen in the present study. Duet applied with the Swingfog provided rapid and widespread control despite sub-optimal conditions, although uneven terrain led to longer spray time in that instance. Prior studies in hot-arid areas in California had shown thermal fog applications superior to ULV when using Fyfanon against mosquitoes, but the present trials showed the reverse against sand flies.

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DIVERSITY AND COMPOSITION OF ANTHROPOPHILIC SPECIES OF *ANOPHELES* (*DIPTERA: CULICIDAE*) IN TWO MALARIA ENDEMIC AREAS OF COLOMBIA

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A central theme in community ecology is the understanding of the factors driving species composition, diversity and variation. It is more important to determine how diversity and composition vary among sites than the number and identity of species in a given site. Therefore, we sought to provide updated information on the diversity, composition and geographical distribution of anthropophilic *Anopheles* species in six localities in Colombia, in the Urabá-Bajo Cauca-Alto Sinú (UCS) and Pacific (P) regions. Each locality was visited four times, every three months,

from November 2008 to June 2010. Mosquitoes were collected for five consecutive nights (18:00-24:00 h), and one additional night (18:00 to 6:00 h). A total of 9,839 specimens belonging to 10 species were collected. Relative abundance showed a reverse-J-shaped curve with few dominant and many rare species. In the overall survey, *An. nuñeztovari* s.l. and *An. darlingi* were the most abundant (47.21% and 40.47%, espectively). *An. punctimacula* and *An. neivai* (<0.01%) were the least bundant. *An. nuneztovari* s.l. predominated mainly in UCS (84.53%), whereas *An. darlingi* dominated in P (55.14%). Other species were found in only one area and in low abundance; *An. pseudopunctipennis* (4.72%), *An. albitarsis* s.l. (2.62%) and *An. triannulatus* s.l. (1.96%) in UCS, and *An. calderoni* (10.63%) and *An. albimanus* (3.63%) in P. UCS had the highest anthropophilic anopheline diversity. Diversity values at different spatial and temporal scales showed statistically significant differences, suggesting that anopheline communities present complex dynamics. In general, the composition, abundance and diversity of these anophelines were highly variable; therefore, control programs should be adapted to the characteristics of each community for maximal efficiency.

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TECHNIQUE FOR PRESERVATION OF MICROFILARIAE OF *WUCHERERIA BANCROFTI*

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Five different types of anti-coagulant, namely, heparin, ACD, ACD-D5, CPD and CPDA-1, were studied for their abilities in preserving microfilariae of *Wuchereria bancrofti* in blood collected from infected patients. The rates of infection generally decreased as the duration of preservation increased. There was no infection detected in mosquitoes fed with blood containing heparin, ACD and ACD-D5 on day 8, day 10 and day 9 after blood collection, respectively. As for mosquitoes fed with blood containing CPD and CPDA-1, infections occurred even the blood used was 10 days old after collection. The responses of mosquitoes to feeding of patients' blood with five different anti-coagulant formulae did not differ significantly on each day from day 3 to day 10 after collection ($P>0.05$). The average numbers of the third infective stage larvae of *W. bancrofti* per mosquito fed with patients' blood with five different anti-coagulant formulae ranged from 0.3 to 3.4 larvae per mosquito.

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A SURVEY OF *ANOPHELES* SPECIES IN A MALARIA ENDEMIC AREA ALONG THE THAI-MYANMAR BORDER

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Anopheles larval mosquitoes were surveyed from the stream in Kanchanaburi Province, western Thailand over a two-year period. Three major groups were morphologically identified, including Minimus subgroup and two other related species (75.74%), Maculatus group (20.47%), and Barbirostris group (0.48%). The other 116 specimens were morphologically identified as *An. culicifacies* (3.05%), *An. philippinensis* (0.17%), and *An. vagus* (0.09%). Based on a molecular identification assay, 2 species within the Minimus subgroup were identified, *An. minimus* (69.93%) and *An. harrisoni* (0.06%) and 2 genetically related species within the Aconitus subgroup, *An. aconitus* (0.63%) and *An. varuna* (5.13%) were described. The Minimus subgroup and other related species were more prevalent during the dry season (52.58%) compared to the hot and rainy seasons. In general, number of *Anopheles* larvae collected from the stream was significantly higher in the second year compared to the first year. This study suggested that site-specific studies should be conducted to accurately determine vector larval habitats and adult activity patterns and linking their importance in malaria transmission in a given area.

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INSIGHTS IN *WOLBACHIA* - TSETSE (GENUS *GLOSSINA*) SYMBIOTIC INTERACTIONS

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Wolbachia is a genus of endosymbiotic α-Proteobacteria infecting a wide range of arthropods and filarial nematodes. *Wolbachia* is able to induce reproductive abnormalities such as cytoplasmic incompatibility (CI), parthenogenesis, feminization and male killing, thus affecting biology, ecology and evolution of its hosts. The bacterial group has prompted research regarding its potential for the control of agricultural and medical disease vectors, including *Glossina* sp., which transmits African trypanosomes, the causative agents of sleeping sickness in humans and nagana in animals. In the present study, we employed a *Wolbachia* specific *16S rRNA* PCR assay to investigate the presence of *Wolbachia* in six different laboratory stocks as well as in natural populations of eleven different *Glossina* species originating from 11 African countries. *Wolbachia* was prevalent in *Glossina morsitans morsitans*, *G. morsitans centralis* and *G. austeni* populations. It was also detected in *G. brevipalpis*, and, for the first time, in *G. pallidipes, G. palpalis gambiensis, G. p. palpalis and G. medicorum*. On the other hand, *Wolbachia* was not found in *G. fuscipes fuscipes*, *G. m. submorsitans* and *G. tachinoides*. *Wolbachia* infections of different laboratory and natural populations of *Glossina* species were characterized using *16S rRNA*, the *wsp* (Wolbachia Surface Protein) gene and MLST (Multi Locus Sequence Typing) gene markers. This analysis led to the detection of horizontal gene transfer events, in which at least four *Wobachia* genes (*16S rRNA, ftsZ, fbpA* and *wsp*) were inserted into the tsetse fly nuclear genome. In addition, it was shown that *G. m. morsitans* males present higher *Wolbachia* load than females. *Wolbachia* infections were detected in both laboratory and natural populations of several different *Glossina* species. The characterization of these *Wolbachia* strains promises to lead to a deeper insight in tsetse-*Wolbachia* interactions, which is essential for the development and use of *Wolbachia*-based biological control methods.

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SPECIES COMPOSITION OF THE MOSQUITO FAUNA OF WESTERN UGANDA

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The mosquito species composition for several locations in Uganda was described during routine arboviral surveillance and outbreak investigations from the mid 1930s to the early 1970s. During that period, mosquito species of Bundibugyo district (then Bwamba County), where Semliki Forest is located, were investigated in detail and over 160 mosquito species were described in this region. Civil instabilities in the 1970s and 1980s halted routine arboviral disease investigations and mosquito species records in Uganda have not been updated for more than 40 years.

During recent arboviral surveillance and zoonotic disease investigations in western Uganda conducted by Uganda Virus Research Institute and the US Centers for Disease Control and Prevention (CDC), mosquitoes were collected in five locations in western Uganda: Sempaya, in Semliki Forest, Kibale Forest, Bwindi Impenetrable Forest, and Mweya and Maramagambo Forest in Queen Elizabeth National Park. Seventy-three species were identified in Sempaya including five species described in Bundibugyo District for the first time: *Aedes (Stegomyia) aegypti formosus* (Walker), *Aedes (Stegomyia) metallicus* (Edwards), *Anopheles (Cellia) rivulorum* Leeson, *Uranotaenia (Uranotaenia) chorleyi* Edwards and *Uranotaenia (Uranotaenia) pallidocephala* Theobald. Twenty-eight mosquito species were identified in Kibale Forest, 41 in Bwindi Impenetrable Forest, 36 in Mweya and 51 in Maramagambo Forest. This is the first description of the mosquito fauna for these four locations. Mosquito species composition and the implication for arboviral transmission for these five locations will be discussed.

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TARGETING THE *PHLEBOTOMUS PAPATASI* PPCHIT1 AS A STRATEGY TO CONTROL SAND FLY-TRANSMITTED LEISHMANIASIS

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For sand flies, the peritrophic matrix constitutes a barrier against *Leishmania* infection. Recently, we have demonstrated that this barrier could be reinforced by knocking down PpChit1, a midgut-specific chitinase of the sand fly *Phebotomus papatasi*, pointing to PpChit1 as a target for paratransgenesis and transmission blocking vaccine approaches. As anti-PpChit1 antibodies are capable of neutralizing chitinolytic activities in midgut extracts not only of *P. papatasi*, but also of *P. argentipes*, PpChit1 may be a target in cross-species strategies against leishmaniasis. In order to assess the potential of anti-PpChit1 antibodies reducing *Le. major* load in *P. papatasi*, we tested two different anti-PpChit1 antisera: anti-full length and anti-catalytic domain PpChit1antisera. Recombinant VR2001 plasmids encoding full length and PpChit1 catalytic domain were injected in mice ears four times in two weeks intervals. Specificity of such antisera was tested by Western blots against midgut extracts, the respective PpChit1 recombinants, and recombinant peritrophins. The recombinant proteins were produced in CHO-S mammalian cells and purified with Ni-NTA columns. Sand fly infections were performed with 5 x 10^6 amastigotes/ml along with neat anti-PpChit1 serum or pre-immune serum. Contrasting to our previous results on PpChit1 knock down via RNAi, feeding on anti-full length PpChit1 antiserum increased *Le. major* load in *P. papatasi* midguts. As this antiserum cross-reacted with other midgut extract proteins displaying the expected sizes of peritrophins and with the recombinant peritrophins, the cross-reactivity may be affecting *Le. major* development in an unexpected manner. Antiserum against the PpChit1 catalytic domain, on the other hand, recognized a single band in midgut extracts and the recombinant catalytic domain of PpChit1, suggesting it specifically recognizes PpChit1. We are currently testing if this antiserum can reduce *Le. major* load in *P. papatasi*. Future work is planned to assess if anti-PpChit1 catalytic domain antiserum also can reduce transmission to naïve hosts.

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CONTAMINATION OF *ANOPHELES ARABIENSIS* WITH PYRIPROXYFEN USING ODOR BAITED STATIONS

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Mosquito larviciding is expected to play a key role in malaria control strategies alongside targeting the adult vectors and malaria parasite. It was recently shown that vectors of Dengue fever can be used to disseminate pyriproxyfen (PPF), a juvenile hormone analogue, into their own breeding sites. For this process to be successful, wild mosquitoes must pick up the larvicide and retain it until reaching a breeding site, where during the oviposition process, they contaminate the water body. This study aimed to adapt the technique for malaria vectors in rural Tanzania. Odor baited stations (OBS) have been tested in this area and were successful in attracting large numbers of wild mosquitoes. PPF powder was applied to the eave-baffles of OBS which served as contamination sites for wild mosquitoes. Three trap nights were conducted and each morning the mosquitoes were collected from exit traps mounted on the OBS. Species collected consisted of both Anophelines and Culicines. These mosquitoes are known to share breeding sites in the dry season in this area, making them potential PPF carriers for malaria control. Collected mosquitoes were then killed and dipped in cups of water containing 10 *Anopheles arabiensis* larvae. Reduction in mosquito emergence was observed from these cups and emergence decreased as the number of contaminated mosquitoes was increased in the cups. Further studies are being carried out to test whether cows can be sprayed with PPF in order to contaminate zoophagic mosquitoes during blood feeding.

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IRON-BINDING PROTEINS OF THE SCABIES MITE *SARCOPTES SCABIEI* AND THEIR IMPLICATIONS FOR INFECTION

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Scabies is a neglected parasitic disease with debilitating effects for both humans and animals. Secondary infections with bacteria are common in crusted scabies patients, particularly in remote Aboriginal communities. It has been reported that pigs can develop auto-antibodies to transferrin during infection with *Sarcoptes scabiei*, where the majority of pigs infected with *S. scabiei* tested positive for both IgG and IgM antibodies to transferrin. Research suggests that this may also be the case for human scabies patients. Recognition of commercial human transferrin by human IgG has been shown by preliminary ELISAs for both crusted and ordinary scabies. It is presumed that a similar mechanism is involved for the production of auto-antibodies in both humans and pigs. A *S. scabiei* var. *hominis* EST database was used to screen for possible iron-binding homologues to transferrin and ferritin. Transferrin was expressed in bacteria as two single domains, N- and C-terminals. Ferritin was expressed as a whole protein. Bioinformatic analysis revealed that mite transferrin has lost many of the conserved residues required for binding iron at both terminals. A pig model was developed by scientists at QIMR for human scabies research. Analysis of recombinant proteins by ELISA indicated that mite iron-binding proteins are antigenic to experimentally infected pigs but not naïve pigs. Competition ELISAs suggest that the antibody response to host proteins may be the result of cross-reactivity with mite epitopes. Further testing using human antibodies to detect recombinant mite proteins is required to determine their antigenicity to humans. The possible loss of iron-binding ability by mite transferrin may have implications for the infection process in both humans and animals.

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ARTIFICIAL LIGHT INCREASES HOUSE INFESTATION BY NON-DOMICILIATED *TRIATOMA DIMIDIATA*

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Triatoma dimidiata is a major vector of Chagas disease and we previously documented the seasonal infestation of houses by this species in the Yucatan peninsula. We also found that bugs were specifically attracted to houses, but the factors mediating this attraction remained unclear. Artificial light has been known for a long time to attract many insect species and light traps have been used to collect different species of triatomines, including *T. dimidiata*. Several authors have also suggested that light might attract *T. dimidiata* to houses, but the role of artificial light in house infestation has never been clearly demonstrated or quantified. Here we performed an exhaustive spatial analysis of house infestation pattern by *T. dimidiata* in relation to the distribution of artificial light sources in three different villages from the Yucatan peninsula. In all three villages, infested houses were on average significantly closer to artificial light sources than non-infested houses, and public lights rather than domestic lights were associated with house infestation. Thus, houses closer to a public street light were 2.72 times more likely to be infested that houses further from public lights (OR, CI95% 2.04-3.61). Behavioural experiments using a dual-choice chamber further confirmed that adult *T. dimidiata* is attracted to white light during its nocturnal activity and in a dose-response manner. While public lighting is usually associated with increased development, these data clearly show that it also directly contributes to house infestation by non-domiciliated *T. dimidiata*.

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CORRELATION OF ANTIBODY AVIDITY WITH DENGUE DISEASE SEVERITY IN HUMAN SERUM SAMPLES

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Dengue virus (DENV) continues to be a major health problem in tropical and subtropical regions worldwide. A secondary infection with a different DENV serotype is a risk factor for severe disease, and the humoral and cellular immune response have been implicated in both protection and pathogenesis. Avidity is a measure of the overall strength of antibody-antigen interaction and depends on the number and affinity of individual binding sites. We hypothesized that low-avidity anti-DENV antibodies could be associated with greater occurrence of symptomatic infection or more severe disease. We are analyzing well-characterized serum samples from a hospital-based study of pediatric dengue in Managua, Nicaragua, ongoing since 2005. Serum avidity was measured using a modified competition ELISA protocol. Purified viral particles (Nicaraguan strain DENV2 N172) were used as antigen. Serum from primary and secondary DENV infections diluted in blocking buffer were pre-incubated with DENV2 for 1 hour ("competition"), then virus-coated wells were incubated with the serum-virus mixtures, followed by the secondary antibody and substrate. For each plate, background values were subtracted, then the percent of inhibition of antibody binding was calculated by subtracting the ratio of the adjusted OD after virus competition from the adjusted OD without virus competition. Plates were normalized using WHO anti-DENV polyvalent reference serum. The assay was validated by confirming higher avidity IgG antibodies in DENV-immune serum against viral particles of the same serotype in comparison to DENV viral particles of a different serotype. Also, avidity was greater in cases of secondary DENV infections compared to primary infections, as expected. Preliminary results of analysis of secondary DENV2 cases from the hospital-based study (dengue fever, n=12; dengue hemorrhagic fever, n=10; dengue shock syndrome, n=13) revealed a trend of inverse correlation between serum avidity and disease severity. This trend was also detected using a different avidity ELISA protocol with urea washes. These results are being correlated with homotypic and heterotypic neutralization/enhancement titers. Overall, these studies should help define antibody characteristics associated with mild versus severe dengue disease useful for vaccine development as well as furthering our understanding of dengue pathogenesis.

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IN COLOMBIAN OUTPATIENTS WITH CLINICAL SUSPICION OF DENGUE FEVER, HOST BIOMARKERS DIFFERENTIATE BETWEEN SUBJECTS WITH CONFIRMED DENGUE FEVER AND LEPTOSPIROSIS

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Dengue represents the most important arboviral infection worldwide and is of increasing global importance. Causes of fever are often non-specific. Acute febrile syndromes like dengue fever and leptospirosis have overlapping geographic distributions, similar clinical presentations and potentially life-threatening complications, but require different treatments. This study was undertaken to determine if perturbations in host biomarkers can differentiate between individuals with dengue fever and leptospirosis during the acute phase of illness. We randomly selected subjects from a prospective cohort study of acute febrile illness in Bucaramanga, Colombia and tested 18 serum biomarkers by ELISA in individuals with dengue fever (DF, n=112) and leptospirosis (n=47). Biomarkers were selected for further analysis if they had good discriminatory ability (area under the ROC curve (AUC) >0.80) and were beyond a reference range (assessed using local healthy controls). We identified 5 candidate biomarkers (Ang-like 3, IL-18 binding protein (IL-18BP), IP-10, sICAM-1, sEndoglin) that were dichotomized (based on the Youden index) to create a score ranging from 0-5. Using this biomarker score, we could discriminate between dengue and leptospirosis with an AUC of 0.96 (95% CI, 0.93-0.99). In order to generate a more parsimonious biomarker score, we took the 2 biomarkers with the best discriminatory ability (AUC >0.90, IL-18BP and sEndoglin). We added the 2-biomarker score to an easy-to-measure bedside index consisting of the 3 clinical predictors with the best discriminatory ability (assessed using forward step-wise regression): sore throat, facial erythema and hepatomegaly. The bedside index on its own had moderate discriminatory ability (AUC, 95% CI; 0.74, 0.67-0.81) whereas a bedside index in conjunction with biomarkers had excellent discriminatory ability (AUC, 95% CI; 0.95, 0.90-0.98), and was significantly better than the bedside index alone ($p<0.0001$, Method of Delong et al.). In conclusion, these results suggest that host biomarkers may have utility in differentiating dengue fever from leptospirosis.

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CHARACTERIZING INTRA-HOST DIVERSITY OF DENGUE VIRUS POPULATIONS AND HOST IMMUNE EFFECTOR REPERTOIRES IN HUMAN DENGUE VIRUS INFECTIONS

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Dengue virus (DENV), a positive-strand RNA *Flavivirus*, causes tens of millions of cases of dengue annually, with three billion people at risk for infection worldwide. The clinical manifestations of dengue range from subclinical infection to life-threatening syndromes, and despite intense research efforts, precise causes for the observed range in disease severity remain unclear. We are employing high-throughput sequencing to investigate the contribution of diversity associated with viral populations and host immune effectors to DENV evolution and the course of dengue pathogenesis. This study utilizes samples from two long-term studies of pediatric dengue in Nicaragua, accompanied by extensive supporting clinical and epidemiological data. Our DENV sequencing efforts are the first to showcase the diversity landscape across the entire DENV genome in human samples and are revealing relationships between INTER-host diversity (i.e., across individuals and epidemics) and INTRA-host diversity (i.e., within an individual), with implications for the study of DENV evolutionary dynamics. We are also simultaneously assessing longitudinal differences in B cell diversity, as assessed by immunoglobulin heavy chain rearrangements ("*IgH*"), for an unbiased evaluation of the clonality of infection-associated antibodies in blood during DENV infection. We have identified expansion of B cells with specific *IgH* in unsorted PBMCs during primary and secondary DENV infections, with clones from this initial cohort appearing earlier for patients who present with secondary infections, as would be expected if there were contributions from pre-existing immunity. We hope that our analyses of samples for which we have both types of datasets available will allow us to discern correlations between hotspots for viral diversity (or immutable regions), and specificity of the antibody response (i.e., expansion of specific B cell clones, as assessed by diversity of B cell-associated *IgH*). Such correlations may facilitate assessment of whether regions of diversity or regions of immutability serve as potential protein epitopes for engaging B cell-directed immunity.

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CHARACTERIZATION OF THIRD AND FOURTH DENGUE VIRUS INFECTIONS IN A PEDIATRIC COHORT STUDY

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Four dengue virus serotypes (DENV1-4) circulate globally, causing more human illness than any other arthropod-borne virus. DENV infection results in inapparent infection, Dengue Fever, life-threatening DHF/DSS with fluid loss and hypotensive shock, or other severe manifestations. The immune response to DENV protects against re-infection with the same serotype; however, the greatest risk factor for severe disease is prior infection with a heterologous DENV serotype. Yet, little information exists specifically about 2nd vs 3rd vs 4th DENV infections, which are all classified as secondary (2°) infections. A cohort study of dengue established in Managua, Nicaragua, in August 2004 is now in its 7th year, following 3,700 children 2-14 years old, with ~5% annual loss to follow-up. Participants are encouraged to come to the study health center at first sign of illness, and 94% of febrile illnesses present within 72 hours of symptom onset. A healthy annual blood sample is collected, and paired annual samples are examined on a yearly basis to identify new primary and 2° infections. Primary infections are defined as seroconversions and 2° infections as a $\geq$4-fold increase in anti-DENV antibody (Ab) titers, determined by Inhibition ELISA. Using data from 7 dengue seasons and 6 annual sample collections, we identified 16 children who entered the cohort DENV-naïve and experienced 3 documented DENV infections; 103 who entered with anti-DENV Abs and had 2 additional DENV infections, and 4 who entered with anti-DENV Abs and had 3 documented infections. We are currently investigating these repeat infections in serial annual serum samples by determining the endpoint neutralizing titer (NT_{50}) to the 4 DENV serotypes using a flow cytometry-based neutralization assay. A separate analysis of DENV2 DHF/DSS cases in the cohort revealed that the majority (5/9; 56%) had evidence of multiple prior infections (substantial NT_{50} titers to DENV1 and DENV3); in contrast, only 6/29 (21%) of DF cases caused by DENV2 had high DENV1 and DENV3 titers prior to infection. The results of confirmed 3rd and 4th infections in relation to infection outcome (symptomatic vs inapparent), disease severity, interval between infections, and sequence of DENV serotypes will be presented. Further studies will compare these results to those obtained in 2nd DENV infections. These data should advance our specific knowledge of repeat DENV infections, with implications for vaccine design.

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ACUTE TRANSAMINITIS DURING DENGUE FEVER ILLNESS

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Dengue fever is endemic to Singapore, where it mainly affects the adult population. Acute transaminitis, or increase in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) liver enzyme levels, has commonly been observed in dengue patients during early illness. However, there are few studies showing the relationship between acute transaminitis and disease severity. 699 dengue PCR positive cases seen at Tan Tock Seng Hospital in Singapore from 2006 to 2008 were retrospectively reviewed, and demographic, clinical, laboratory, and disease outcome data were extracted. Statistical analyses were performed to show any association between AST or ALT and disease outcomes. 86.4% of cases had AST levels greater than the upper limit of normal (ULN), and 46.4% had ALT above ULN. Only 10 patients had AST or ALT >1000 U/L, fulfilling the 2009 World Health Organization (WHO) definition of severe liver impairment. Median AST was 92 U/L for non-severe dengue cases and 125 U/L for severe dengue patients (WHO 2009 classifications), excluding those with isolated transaminitis--one of the severe dengue criteria ($p<0.005$); median ALT values were 52 and 74 U/L ($p<0.005$). AST and ALT values also increased significantly in conjunction with disease severity under the WHO 1997 classification system. 3 patients required intensive care, and 1 died. AST or ALT values did not correlate with hospital length of stay (Spearman's rho=-0.02 and -0.03; p=0.59 and 0.37, respectively). Regression analysis showed that determining an AST or ALT cutoff to predict severe dengue was difficult since the area under the receiver operating characteristic (ROC) curve was 0.62 for AST and 0.59 for ALT. Most of the dengue cases in this study experienced transient transaminitis. There was a significant overall association of higher AST and ALT levels with severe disease outcomes, but there was no good cutoff level to predict severe dengue.

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SPATIOTEMPORAL PATTERNS OF *AEDES AEGYPTI* MOSQUITO POPULATIONS IN CAIRNS: ASSESSING THE DRIVERS OF RISK

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Understanding the dynamics of the primary dengue vector, *Aedes aegypti*, is vital to controlling the disease. Current monitoring in Cairns, Australia, involves trapping *Ae. aegypti* mosquitoes in sticky Ovitraps, with weekly mosquito counts determining whether adult mosquito abundance is within expected limits. This study aimed to determine relationships between *Ae. aegypti* and environmental drivers: rainfall, temperature and humidity. Data from sticky Ovitraps traps in Cairns were collected for the period 2007-2010. Climate data (rainfall, temperature and humidity) for Cairns were accessed via the Bureau of Meteorology for the same period. Exploration of the data was undertaken using Spearman's rank correlation and Poisson regression. Results showed positive correlations between *Ae. aegypti* abundance and maximum weekly temperature (rho = 0.5342), total weekly rainfall (rho=0.3178) and average weekly humidity (rho=0.3844). Maximum weekly temperature was a statistically significant predictor of *Ae. aegypti* abundance (p= 0.000). The expected change in log count for a one degree Celsius increase in temperature was 0.1830. Total weekly rainfall was also a statistically significant predictor of *Ae. aegypti* abundance (p= 0.028). The expected change in log count for a one millimeter increase in rainfall was 0.0020. Interestingly, humidity was not a significant predictor of *Ae. aegypti* abundance (p= 0.0700). Dengue is an emerging arbovirus that causes considerable morbidity and mortality in the Asia-Pacific region. This study contributes information on the influence of environmental drivers (rainfall, temperature and humidity) on *Ae. aegypti*, the primary dengue vector. Findings suggest that control of the vector should be focused on climatic factors because abundance of *Ae. aegypti* is associated with these conditions. This information can be used with other data to predict the risk of dengue in a given geographic location.

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SAFETY PROFILE OF THE CYD LIVE, ATTENUATED, TETRAVALENT, DENGUE VACCINE

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A tetravalent dengue vaccine (TDV) comprising 4 recombinant, live, attenuated viruses, (CYD-1-4), given with a 3-dose regimen over 12 months, is currently in clinical phase 3 evaluation. We performed an integrated analysis of safety of all 13 trials (3 phase I, 5 completed phase II, and 5 ongoing phase II trials) conducted to date with the current TDV formulation, in both dengue-endemic and non-endemic areas. Data were analyzed by treatment given for unblinded trials (TDV, placebo, or control vaccine) or in a blinded manner for ongoing trials, and by age group: adults (>/=18 yrs), adolescents (12-17 yrs), children (2-11 yrs), and toddlers (<2 yrs). After the 1st, 2nd and 3rd vaccinations respectively, the TDV analysis set comprised 890, 710 and 555 participants, and the blinded analysis set comprised 6782, 5588, and 3897. After the 1st TDV dose, 21.3% of adults, 24.2% of adolescents, 25.4% of children and 18.3% of toddlers had solicited injection site reactions. These percentages ranged from 20-35% after the 2nd, and 17-30% after the 3rd dose, respectively. In comparison these rates after the 1st injection ranged from 27-56% after active control vaccination, 7-19% after placebo, and 17-37% in the blinded dataset. Solicited systemic reactions after the 1st TDV dose affected 56.2% of adults, 63.7% of adolescents, and 45% of children and toddlers, decreasing to 28-48% after the 2nd, and 20-37% after the 3rd. In comparison, after the 1st injection these rates ranged from 39-73% after active control vaccination, 29-49% after placebo, and 40-55% in the unblinded dataset. Overall, headache was the most frequent solicited systemic reaction after any TDV dose. Most solicited systemic reactions were mild and lasted 1-3 days. The incidence of serious adverse events was similar in the TDV group compared with other groups. Reactogenicity was no higher in children than adults, and no higher after the 2nd or 3rd doses compared with the 1st. Based on all available data, TDV has a satisfactory safety and reactogenicity profile, comparable to that of the control vaccines.

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2011 UPDATE ON THE SANOFI PASTEUR CYD TETRAVALENT DENGUE VACCINE: INITIATION OF CLINICAL PHASE 3 PROGRAM

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While no licensed vaccine is available against dengue, the sanofi pasteur 2nd generation candidate, in development since 1998, has reached industrial scale-up, clinical phase III, and has been given to more than 6000 children and adults. This candidate, a mixture of 4 recombinant, live attenuated viruses (CYD-1-4) at 10^5 $CCID_{50}$/virus, is being tested with a robust 3-dose, 0-6-12 month regimen. Industrialization efforts include the construction of 3 new dedicated facilities (Utilities, QC and Production) at a new vaccine production site in Neuville-sur-Saone, France. Banking systems for serum-free Vero cells have been established to produce master and working viral seeds and cells, providing reliable and consistent supply, a production process with no raw materials of animal origin, and a vaccine with no preservatives, adjuvants, or antibiotics. To further characterize the vaccine's safety, a biodistribution and shedding study was performed in cynomolgus monkeys, showing that there was no neurotropism, no shedding, and only limited viscerotropism; CYD viruses were limited mainly to the injection site and the lymph nodes during the first days after injection and were not associated with any toxicological findings other than expected injection site reactions. An integrated safety analysis of all available data from completed phase 1 and 2 studies, and on blinded data from all ongoing phase 2 studies shows a satisfactory safety and reactogenicity profile, comparable to that of control vaccines. The 1st phase 3 trial started in 2010 in Australian adults to test lot consistency. Among several phase 3 trials due to start in 2011, 2 are efficacy studies conducted in 2-16YO children from multiple countries in Asia and Latin America respectively. To prepare the sites for these trials, in particular to assess whether potential dengue cases can be identified and to test the diagnostic algorithm in the field, prospective, active-surveillance studies were initiated in these areas. Results from an ongoing efficacy proof-of-concept trial are expected by the end of 2012.

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IMMUNOGENICITY AND LARGE SCALE SAFETY OF THE LIVE, ATTENUATED, TETRAVALENT, CYD DENGUE VACCINE IN 2-45 YEAR-OLDS IN SINGAPORE

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A tetravalent dengue vaccine (TDV) comprising 4 recombinant, live, attenuated viruses (CYD-1-4) is currently in clinical phase 3 evaluation. In an observed-blinded, age-stratified study, we randomized 1200 volunteers 3:1 to receive 3 subcutaneous doses of TDV or control at Months 0-6-12 (ClinicalTrials.gov: NCT00880893). Controls were placebo for the 1st dose (all ages) and licensed hepatitis A (for <12YO) or influenza vaccine (≥12YO) for subsequent doses. The primary objective was to evaluate dengue virus (DENV) serotype-specific antibody responses before and 28 days after each vaccination in a subset of 600 using a $PRNT_{50}$ assay. Safety of TDV was documented as a co-primary objective. Between Apr and Oct 2009, we enrolled and randomized 317 children (2-11YO), 187 adolescents (12-17YO), and 696 adults (18-45 YO). At baseline ~10% of children/adolescents and ~30% of adults were seropositive (titer ≥10), to at least one serotype. After 3 TDV doses, 66.5% (all ages combined) were seropositive to all 4 serotypes, and 87.2% were seropositive to ≥3 serotypes. Geometric mean titers 28 days after the 3rd dose of TDV (all ages) ranged from 43.0 against DENV1 to 100 against DENV4. Titers were higher against in children than in adolescents. Titers in the control group remained close to baseline levels. The 1st dose of TDV was slightly more reactogenicity compared with placebo. Reactogenicity of subsequent TDV doses was no higher than after the 1st, and was comparable with that of the active control vaccines. One SAE (tension headache secondary to untreated allergic rhinitis 17 days after 2nd dose in a 9YO boy) was reported as possibly-related to TDV by the investigator. The safety profile of CYD TDV in a large cohort of volunteers from Singapore was satisfactory and consistent with observations from earlier trials. Vaccination elicited an immune response against all 4 serotypes in the majority of vaccinees. Some differences in response between age group were noted, possibly reflecting the unique epidemiology of dengue in Singapore.

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ANALYSIS OF *IN VITRO* ENHANCEMENT RESPONSE CURVES AGAINST INFECTED PATIENT AND VACCINEE SERA

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Antibody-dependent enhancement (ADE) has been correlated with increased dengue disease severity. However, measurements of ADE *in vitro* can be dependent on the experimental and analytical parameters used, and their relevance to quantification of ADE and clinical outcome has not been well studied. Here we used pseudoinfectious Dengue virus (DENV) reporter virus particles (RVPs) from each of the four serotypes of DENV to measure and characterize the enhancing response of monoclonal antibodies and sera derived from naturally-infected patients and tetravalent dengue vaccine recipients. Enhancement assays were done in multiple cell types, using various input titers of virus, and under different experimental conditions to derive detailed enhancement curves. Several distinct types of enhancing curves were observed, and a curve fit equation was derived for each type of curve in order to calculate precise enhancement titers. Three distinct metrics were derived from each enhancement curve - titer (peak serum dilution), power (peak height), and polydispersity (peak width) - and the effect of experimental conditions on each metric was assessed to determine the most reliable indicator of *in vitro* enhancement (i.e. independent of experimental conditions). To help determine the clinical relevance of ADE measurements, each measurement was also correlated with neutralization titers derived from each serum (against all 4 serotypes) as well as clinical outcomes of patients and vaccinees. A better understanding of *in vitro* ADE measurements may help quantify the potentially protective and pathogenic immune responses generated against each serotype of DENV within infected and vaccinated patients.

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RISK FACTORS FOR FATALITY AMONG CONFIRMED ADULT DENGUE INPATIENTS IN SINGAPORE: A MATCHED CASE-CONTROL STUDY

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Dengue is an important viral infection affecting most tropical and subtropical areas of the world. Reports of death in adult dengue cases are rare. We conducted a multi-center retrospective study of polymerase chain reaction and non-structural protein 1 (NS-1) confirmed adult (older than 15 years) dengue inpatients in Singapore from 1 January 2004 to 31 December 2008. Initial unmatched analysis showed age was significantly different among fatal cases and non-fatal controls ($p<0.001$). Subsequent analyses matched for age and year of admission to control for different predominant circulating dengue serotypes and yielded 28 cases and 80 controls. World Health Organization 1997 and 2009 criteria were applied to define dengue hemorrhagic fever (DHF), warning signs and severe dengue. Statistical significance was assessed by conditional logistic regression modeling. Versus controls, fatal cases had significantly more comorbid conditions (75% versus 51.3%; $p<0.01$), renal injury defined as serum creatinine more than two times upper limit of normal (71.4% vs. 2.5%; $p<0.001$), warning signs (96.4% vs. 75%; $p<0.05$), severe dengue (100% vs. 22.5%; $p<0.01$), higher median pulse rate (128 vs. 95 per minute; $p<0.001$), alanine transaminase (490 vs 74 Unit/Liter; $p<0.05$) and aspartate transaminase (1158 vs. 112.5 U/L, $p<0.05$) during hospitalization. Leukocyte count and serum protein were significantly lower among cases ($p<0.001$ and $p<0.05$ respectively). There was no statistical significant difference between the prevalence of DHF, median platelet nadir and hematocrit level among cases and controls. The rates of intravenous fluid and platelet transfusion were higher among cases ([92.9% vs. 41.3%; $p<0.05$] and [64.3% vs. 11.3%; $p<0.01$ respectively]). None of the controls were admitted to intensive care unit (ICU) or given blood transfusion, while 71.4% and 28.6% of cases required ICU admission and given blood transfusion. None of the variables was statistically significant in the multivariate analysis. Findings from this study should be validated by larger cohorts.

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ANALYSIS OF DENGUE VIRUS 3 PRM/E MUTANTS TO DETERMINE THE CONTRIBUTION OF EACH RESIDUE TO ENV FUNCTION

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While the functions of Dengue virus (DENV) prM/E are well known, most functional regions of the protein have only been partially mapped. Using Shotgun Mutagenesis technology, a comprehensive plasmid mutation library for DENV-3 prM/E was created in which each prM/E residue was

individually mutated to a defined substitution, expressed in human cells, and analyzed for its effect on viral production and infectivity. This approach expresses each prM/E mutant in mammalian cells that contain the complementary nonstructural proteins required to produce infectious DENV reporter virus particles (RVPs). By looking for expression of a luminescent reporter in target cells, each mutant's ability to support viral infection can be assessed. In total, over 1,000 mutants of DENV prM/E were individually tested for function. Intermediate functions of prM/E, such as viral budding, were also assessed. Structures that are functionally responsible for viral production and infectivity have been mapped in order to better understand how the protein functions. The identification of critical functional structures is expected to help direct the development of therapeutics, diagnostics and vaccines.

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DENGUE FEVER AMONG HOSPITALIZED FEBRILE PATIENTS IN NORTHERN TANZANIA

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Despite its initial description in the tropics at the 17th century, little is known about the prevalence of dengue (DEN) virus infection in sub-Saharan Africa, particularly during non-epidemic periods. To understand the role of DEN as a cause of illness among febrile inpatients, we conducted a one year prospective study in northern Tanzania from September 2007. Serum collected from febrile inpatients from two hospitals was tested for DEN IgM and IgG antibodies using an IgM capture ELISA and Indirect IgG ELISA (both PanBio, Brisbane, Australia) and for DEN and flavivirus by PCR using previously validated primers. Presumptive acute DEN infection was defined as a positive anti-DEN IgM ELISA result. A positive anti-DEN IgG ELISA defined prior flavivirus exposure. Confirmed acute DEN or flavivirus infection was defined as a positive PCR for DEN or flavivirus, respectively. Of 870 participants, DEN IgM serology was performed on 747 (86.0%); 380 (50.1%) were infants and children, 356 (47.7%) were females, and 326 (44%) lived in rural areas. Seventy one (9.5%) had presumptive acute DEN infection; their median (range) age was 14.4(0.3, 95.8) years. DEN IgG serology was performed on 751(86.3%); 384 (51.1%) were infants and children. Of those tested, 80(10.7%) had prior flavivirus exposure. Unlike presumptive acute DEN infection, prior flavivirus exposure was associated with rural residence, (OR 1.8, p-value 0.027) and was less common among infants and children than among adults and adolescents (OR 0.26, p<0.001). Of participants with presumptive acute DEN, 40 (56.3) had no evidence of an acute co-infection. Among 700 samples tested by PCR, all were negative for DEN and flavivirus. Although we were unable to confirm cases by PCR, serological evidence of infection suggests that DEN or a closely related flavivirus is present in Tanzania. Further research is warranted to identify which flaviviruses are circulating in northern Tanzania, including use of virus isolation techniques.

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ROLE OF FUSION LOOP IN ATTACHMENT OF FLAVIVIRUSES TO HUMAN RED BLOOD CELLS

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The flaviviruses West Nile virus (WNV) and Dengue virus (DENV) are a significant public health burden, infecting millions of people worldwide annually. Both DENV and WNV have been found to attach to human red blood cells (hRBCs) during natural infection without losing infectivity, but the significance of this attachment has not been elucidated. Hemagglutination (HA) experiments show that hRBCs agglutinate WNV at a peak pH of 6.2, with HA decreasing at higher and lower pHs. One possible explanation for lack of HA at clinical pH is the involvement of the fusion loop (FL) of the E protein in hRBC binding. That possibility could be verified by assaying HA with non-infectious immature viral particles, in which the prM protein remains uncleaved by furin, blocking exposure of the FL. With that in mind, we investigated the mechanism of binding of flaviviruses to hRBC and the potential involvement of the FL. We used prototype strains for each of the 4 DENV serotypes and a chimeric virus expressing WNV structural proteins with DENV-4 vaccine strain nonstructural proteins (WNV/DENV-4Δ30), grown with or without NH_4Cl to generate wild-type (WT) or predominantly immature virions, respectively. WT and NH_4Cl-treated stocks were characterized by western blot to detect prM in immature particles, focus-forming assay to quantify infectious virus, and qRT-PCR to quantify total viral RNA from mature and immature particles. The ability of WT and NH_4Cl-treated viruses to agglutinate hRBC was determined in comparison with the concentrations of infectious virus and total viral RNA present in each stock. WNV/DENV-4Δ30 and DENVs 2, 3, and 4 had HA titers proportional to the amount of infectious virus but not to total viral RNA, suggesting that immature virus does not agglutinate hRBCs. NH_4Cl-treatment of DENV1 did not alter the proportion of infectious virus compared to total viral RNA, and thus no conclusions could be drawn. Further studies are underway to quantify binding of wild-type and immature virions to RBCs and further clarify the role of the FL in binding.

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NOVEL VIRUS INACTIVATION PLATFORMS FOR A PURIFIED INACTIVATED DENGUE VIRUS VACCINE CANDIDATE

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A number of vaccine technologies are being explored in the development of a tetravalent dengue vaccine. Inactivated viruses offer a simple and cost effective platform for dengue vaccines. Formalin inactivation could lead to cross linking of surface antigens, thus compromising immunogenicity of the product. We have used photo-inactivation of viruses with two novel reagents that inactivate viruses without perturbing the surface antigenic proteins and compared these with formalin inactivated dengue-2 virus. 1,5-iodonaphthylazide (INA) sequesters exclusively into the lipid bilayer of biologic systems and reacts with membrane domains of proteins when exposed to UV radiation. INA has been successfully used in the inactivation of HIV, VEE and other viruses. 4 -aminomethyltrioxsalen (AMT) is a psoralen that inactivates viruses by reacting with the nucleic acid genome. AMT is a low toxicity drug that is already used in cancer chemotherapy and photo treatment of certain skin disorders. Formalin and INA inactivation of dengue-2 led to substantial (30-50 %) loss of binding to 5 different dengue-2 specific monoclonal antibodies whereas binding of antibodies to AMT inactivated virus was comparable to that of un-inactivated virus. Immunogenicity of various inactivated dengue-2 viruses is being tested in a murine model. After a single inoculation with alum adjuvant, all vaccines elicited antibodies as measured by ELISA. These data and virus neutralizing antibody titers after boosting will be discussed.

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MODELS FOR COMBINED NEUTRALIZATION AND ADE OF DENGUE VIRUS BY TWO MONOCLONAL ANTIBODIES

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Most current research on dengue virus (DENV) neutralization uses either polyclonal serum or single monoclonal antibodies (MAbs). Here we describe studies with defined mixtures of MAbs to quantitatively study

the outcome when more that one type of antibody binds to the DENV particle. For these studies we used a panel of 10 well characterized MAbs representing different IgG subclasses, antigen specificities (E, EDIII, prM), serotype cross reactivity patterns (serotype specific or cross reactive), neutralization potency (strong, moderate, weak), and mechanisms of neutralization (pre versus post attachment neutralization). Our data demonstrate that different MAbs function independently, when present in a mixture. For example, we have not observed any synergistic effects when mixtures of neutralizing antibodies were incubated with DENV. Similarly the ability of antibodies to enhance infection of Fc receptor bearing cells was also strictly additive. Finally we observed that neutralization was dominant over enhancement when pairs of neutralizing and enhancing antibodies co-incubated. Based on these results we will present empirical, mathematical models that predict the neutralization and enhancement properties of antibody mixtures.

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LYMPHATIC FILARIASIS AFTER SELECTIVE TREATMENT IN CENTRAL VISAYAS, PHILIPPINES

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Lymphatic filariasis (LF) is endemic in southern half of the Philippines. This study analyzed the incidence rate of LF in Central Visayas, Philippines (2001-2009) recorded at the Department of Health after selective treatment (albendazole and diethylcarbamazine citrate, DEC, on the first day and by 11-day dose of DEC at 6 mg/kg/day) was given in 2001 or 2002. Incidence rates of LF differed (P< 0.05) among the four provinces in the region for the past nine years. Negros Oriental had the highest incidence rate (0.095 cases/1,000 population), followed by Siquijor (0.074 cases/1,000 population), Bohol (0.0043 cases/1,000 population) and Cebu (0.0006 cases/1,000 population). Overall, LF cases have decreased after selective treatment in Central Visayas, however, slight transmission continued in Negros Oriental. The highest incidence rates of the disease in Negros Oriental were found in Sta. Catalina, Mabinay and Siaton, in that order of rank. LF cases were found in four municipalities in Siquijor (Enrique Villanueva, Larena, Lazi, and Maria), three in Bohol (Dimiao, Loon, and Talibon), and only two in Cebu (Liloan and Toledo).

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BARRIERS TO MASS DRUG ADMINISTRATION IN NORTHWESTERN ARGENTINA: IMPACT OF MIGRATION AND REGIONAL WORK PATTERNS

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Soil transmitted helminthiases (STHs) are among the most prevalent neglected tropical diseases, with the highest burden occurring in impoverished populations. Control of STHs is based on periodic targeted or universal deworming, health education and sanitation. In Orán, located in northwestern Argentina on the Bolivian border, nearly half of the population has unmet basic needs. The sugar cane industry is one of the major employers and the seasonal nature of the work results in significant fluctuations in population numbers. In this study we evaluated the first round of a pilot community-wide mass drug administration (MDA) with ivermectin-albendazole in single doses in a rural village, highly endemic for S.stercoralis and other STHs. Through a cross- sectional survey we assessed drug coverage and causes of non-compliance. Sociodemographic data was extracted from the quarterly census done by the local health services. In the survey conducted in September 2010, the total village population was 618. The first round of MDA, which utilized the Primary Health Care System´s network, was done in December on a house by house basis. It took 3 days to complete the drug distribution. By that time, the sugar cane harvest had been completed and 163 persons had migrated out of the community. Of the remaining 455 persons, 12 met ≥ 1 exclusion criteria, 2 refused treatment, and 120 were missed despite repeated household visits. We were able to treat 321 persons, 74% of the available and eligible population. Of missed person, most were men (80%) of working age (median age: 24 years, IQR 16-41). The analysis of the quarterly censuses confirmed the cyclic variations with the total population increasing by over 30% during the harvest season. Maximum drug coverage is essential in order to have an impact on STH morbidity. In our study we identified 2 major barriers: migration and the local work schedule, which affect preferentially the adult male population. Efforts must be made to determine the best strategies for treating this difficult to-reach population.

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FACTORS ASSOCIATED WITH COMPLIANCE WITH MASS DRUG ADMINISTRATION FOR LYMPHATIC FILARIASIS ELIMINATION IN KENYA: DESCRIPTIVE STUDY RESULTS

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Annual Mass Drug Administration (MDA) to at least 65%-80% of population at risk is necessary for Lymphatic Filariasis (LF) elimination. In Kenya, MDA based on diethylcarbamazine and albendazole, has been implemented thrice in Kwale and Malindi districts. To identify factors influencing compliance with MDA, a retrospective cross-sectional study was conducted in the two districts after 2008 MDA. In Kwale, Tsimba Location was selected for high and Gadini for low coverage while in Malindi, Goshi Location represented high and Gongoni low coverage. Using systematic sampling, nine villages were selected from the four locations. Quantitative data was collected from 965 systematically selected household heads and analyzed using SPSS version 15. For qualitative data, which was analyzed manually according core themes of the study, eighty opinion leaders and eighty LF patients with clinical signs were purposively selected and interviewed and sixteen FGDs conducted with adult and youth male and female groups. Compliance among Christians was higher compared to Muslims ($P< 0.001$). Age, sex and marital status did not influence compliance with treatment ($P> 0.05$). There was a significant difference in compliance with treatment among community members with high income levels and those with low income levels ($P< 0.05$). Compliance was higher among community members who had knowledge of signs, cause of LF and considered themselves to be at risk of LF infection compared to those who did not ($P< 0.001$). Compliance was higher among community members who received information that the drugs were given to treat and control LF than those who did not ($P< 0.001$). There is need for investment in reaching out to groups often missed during MDAs. Different strategies have to be devised to reach specific religious groupings and high income earners. All groups targeted for treatment should be educated about the disease and correct information on MDA relayed to them.

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INNOVATIVE WAYS TO CONDUCT COVERAGE SURVEYS IN MALAWI AND MALI

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Mass drug administrations (MDAs) are used to dispense drugs to populations for preventive chemotherapy for five neglected tropical diseases (NTD). Monitoring of drug coverage is crucial for ensuring that

the goals for control and elimination of NTDs are met and is typically done by monitoring reported coverage. WHO recommends periodic validation using household surveys. We sought to investigate alternative survey methods in an effort to find a quick and resource efficient approach that would provide an estimation of drug coverage. Three alternative survey methods were conducted and compared to the WHO recommended 30-cluster method in one district in Malawi and Mali after a MDA for lymphatic filariasis and soil-transmitted helminths had taken place. For the headman method, a village leader, in 30 villages selected by proportional to estimated size (PPES), was asked to designate a person to survey 10 households. For the religious leader method, a leader of a randomly selected religious establishment, in 30 villages selected by PPES, was asked to designate a person to survey 10 households. For the market method, sub-district markets representing geographic coverage of the district were selected. In each chosen market, a convenience sample of 60 people were surveyed. In Malawi, drug coverage for the 30-cluster, market, headman, and religious methods were 66.8% (95% confidence interval [CI]: 60.3%-73.4%), 74.3% (CI: 71.1%-77.4%), 76.3% (CI: 69.6%-83.0%), 77.8% (CI: 72.5%-83.1%), respectively. In Mali, coverage results were 62.6% (CI: 54.4%-70.7%), 56.1% (CI: 48.8%-63.4%), 74.8% (CI: 65.9%-83.8%), and 83.2% (95% CI: 75.8%-90.6%), respectively. All methods were logistically feasible and accepted by survey participants. Technical errors, such as checking multiple answers to a question or not completely filling out the survey, were noted more often for the headman and religious methods. The market, headman and religious surveys required less resources to complete compared to the 30-cluster survey. The market survey method yielded similar results when compared to the 30-cluster survey. Ways to improve the accuracy of the headman and religious surveys have been identified and will be further tested.

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SCHISTOSOMIASIS AND SOIL-TRANSMITTED HELMINTHIASIS CONTROL IN CAMEROON: PROGRESS MADE, CHALLENGES AND WAYS FORWARD

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Recent years have witnessed an increased interest in the control of schistosomiasis, soil-transmitted helminthiasis (STH) and other so-called neglected tropical diseases (NTD). Taking advantage of this new impetus, Cameroon officially launched its national programme for the control of schistosomiasis and STH in 2004. Starting with very limited budget and no external financial support, the control programme gradually mobilized national and international partners, through intense and multifaceted actions, including advocacy and a number of key achievements. In 2005, Cameroon was selected as the start-up country of Johnson & Johnson's mebendazole donation program because of government leadership and commitment to eliminating infections as a major public health problem. This support enabled a rapid scaling-up of activities to encompass all ten regions in 2007. In its efforts to control these diseases, the government of Cameroon adopted an inter-sector collaboration for the implementation of regular school-based deworming activities. In 2009, the Ministry of Health, the Ministry of Education, and the Union of United Councils and Cities signed an innovative tripartite agreement to capitalize their resources. Furthermore, the NTD control in Cameroon is supported by the USAID/RTI/HKI grant since 2010. Through all this partnership, nearly 6 million children are dewormed annually. More than 75 000 teachers, 14 000 headmasters and health personnel were trained. In 2010 and 2011, mapping was achieved in 7 of the 10 regions of Cameroon to update the distribution of schistosomiasis and STH, and to monitor the programme impact. The results showed a significant decrease of infections in all regions. Over the past 5 years significant progress was made for the control of these diseases, as a result of a coordinated effort of the Government with national and international partners. However, there remain several challenges, including integration with other NTDs. The presentation highlights the achievements, challenges, and ways forward for the control schistosomiasis and STH.

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COVERAGE SURVEYS FOR NEGLECTED TROPICAL DISEASES: TEN YEARS OF FIELD EXPERIENCE

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Mass drug administration (MDA), involving the distribution of preventative chemotherapy to an entire at-risk population, is one of the public health strategies recommended by the World Health Organization (WHO) for the prevention, control and elimination of some neglected tropical diseases (NTDs). Adequate coverage is vital to achieve NTD program goals. Reported coverage is often the main indicator used to evaluate NTD programs. The WHO and several drug donation programs recommend conducting coverage surveys periodically to validate reported coverage and to collect additional information necessary to guide NTD programs. Over the past decade, the CDC and collaborators have conducted more than 20 post-MDA coverage surveys in 7 countries throughout the Caribbean, Africa, and Asia. The method used in each surveyed district was a two-stage 30 cluster household survey. For the first stage sampling, clusters were selected with probability proportional to estimated size while second stage sampling used the 'improved random walk method' to select households for interviews. The survey questionnaires were designed to gather coverage data and information relevant to improving NTD programs including: demographic data, MDA distribution strategies, availability of safe water and sanitation, school-attendance, systematic non-compliance, as well as knowledge, attitudes and practices (KAP) surrounding NTDs. We adapted the questionnaire to be used for both individual and integrated drug packages following MDA conducted with varying distribution strategies: house-to-house distribution, school-based distribution, and distribution posts. After a 2-day training and field testing of the questionnaire, three or four teams of 2 persons conducted a survey in 5-7 days. Feasibility in the field was confirmed by the fact that several countries conducted subsequent coverage surveys with little to no technical assistance. Due to the sampling frame, data don't have to be weighted, simplifying the data analyses. Average cost calculations are ongoing. The main challenge was finding a reliable data source with population figures from all villages to establish a sampling frame; however, with one exception, solutions were found. Our experience has led us to conclude that coverage surveys are feasible to implement and can be adapted to multiple settings and to serve multiple program needs.

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A GLOBAL ACCESS FRAMEWORK FOR ADVANCING TRANSLATIONAL RESEARCH IN NEGLECTED TROPICAL DISEASES

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Neglected infections of poverty afflict more than one-sixth of the world's most marginalized populations and reemergence in the developed world remains a viable threat. Medication toxicities, multidrug resistance, and drug pricing severely limit treatment. The United States National Institutes of Health funds 40% of neglected tropical diseases research worldwide and has taken landmark steps to address the drug innovation crisis by establishing the National Center for Advancing Translational Sciences (NCATS). Providing an equitable access framework to health-related innovations and information may allow the Center to best accomplish its applied research goals. Establishing a global access licensing framework for all technology transfers at NCATS may lead to enhanced dissemination of the discoveries generated. Appropriate models for technology transfer would ideally encourage the acquisition of patents for research products only when necessary to promote commercialization, would utilize non-exclusive licensing agreements, create streamlined processes for materials transferred, and reserve broad rights for the use of patented and licensed technologies for future research. Lessons learned from the University of

British Columbia, which effectively applied these principles to transfer a new amphotericin B formulation for treatment of visceral leishmaniasis to the private sector, could provide a framework for future licensing agreements. Prompt public access to NCATS and more broadly, NTD-related manuscripts, may facilitate information exchange and enhance R&D in rare and neglected diseases. An effective strategy may be to alter the current NIH Public Access Policy to require all investigators receiving NIH funding, including those within NCATS, to submit publically available electronic versions of manuscripts to the National Library of Medicine's PubMed Central within one month of the official date of publication. In conclusion, the policy frameworks proposed aim to improve access to information and technologies generated at NCATS and may be necessary to truly advance the translational sciences.

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DID AN IVERMECTIN MDA REDUCE ENDEMIC SCABIES AND STRONGYLOIDIASIS IN A REMOTE ABORIGINAL COMMUNITY IN AUSTRALIA?

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Scabies and strongyloidiasis are endemic in many Aboriginal communities in northern Australia and contribute to the high morbidity experienced by Aboriginal and Torres Strait Islander people. Previous studies have indicated that both parasite infections can be treated with oral ivermectin. We hypothesized that an ivermectin mass drug administration (MDA) program would be an effective public health measure to reduce prevalence of both scabies and strongyloidiasis in remote settings in Northern Territory, Australia. The project includes a population census for prevalence and MDA conducted at month 0 and 12, and a cross sectional survey at months 6 and 18 to identify disease acquisition and treatment failures. Scabies was diagnosed clinically and strongyloidiasis by parasitology through faecal microscopy and/or agar plate culture or by serology. Participants were administered ivermectin in a dose of 200µg/kg unless pregnant or their weight was <15kg. Those not eligible for ivermectin received 5% permethrin or 10% crotamiton and 200mg or 400mg albendazole daily for 3 days. A second treatment was given to those with a diagnosis of scabies and/or strongyloidiasis within 2-3 weeks of the first treatment. The project commenced in March 2011 enrolling 1011 (81%) participants from 127 (80%) houses and 7 (78%) surrounding homelands. Scabies prevalence reduced from 4% at month 0 to a point estimate of 1.8% at month 6 and strongyloidiasis (predominantly diagnosed serologically) from 21% to 6% over the same period. At month 6, disease acquisition of scabies was 1% and strongyloidiasis 3%, with treatment failures of 11% and 16% respectively. The second population census and MDA#2 is currently underway and preliminary results will be presented at the meeting. The study is due to be completed later this year but the early indications for the success of a strategy incorporating mass treatment for both endemic parasitic infections using the one medication are encouraging and could have national and global implications for informing public health programs and treatment guidelines.

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MATERNAL AND CHILD HEALTH IN NORTHERN ANGOLA: MALARIA, SCHISTOSOMIASIS, GEOHELMINTHS, ANEMIA AND MALNUTRITION IN A POST-WAR SETTING

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Parasitic diseases are an important cause of morbidity and mortality worldwide. In Angola, malaria, schistosomiasis and soil transmitted helminth (STH) infections are endemic diseases. National prevalence surveys were conducted in 2007 and 2005, but no detailed updated information exists. The aim of this study was to determine the presence of malaria, schistosomiasis (urinary and intestinal), and STH infections among pre-school (<6 years old), school-aged (6-15 year old) children and their mothers or caretakers in rural and peri-urban areas in Northern Angola (Dande Municipality, Bengo Province). Furthermore, prevalence levels of anaemia and malnutrition were also assessed. We conducted a community-based random sampling survey, between May and August 2010, which included 36 of the 69 hamlets within the CISA Project Demographic Surveillance System (DSS) study area. In total, 972 households were included, representing 960 mothers and their 2379 children (≤15 year olds). Malnutrition and anaemia were found at elevated levels and should be considered severe public health problems, with a total of 21.4% of children being underweight, a prevalence of chronic malnutrition of 32.2% and anaemia reaching 56.9% among under fives. Malaria prevalence in children was close to 18%, and varied heavily according to geographical location, with some hamlets reaching levels above 50%. Similarly, prevalence levels of urinary schistosomiasis depended heavily on location, reaching an overall prevalence of 16.6% in school-aged children. Finally, STH infections were common, with a prevalence of 31.6% in school-aged children. Information gathered during this study will augment previous work by government initiatives and will provide concrete prevalence levels and causal factors for these infections, anaemia and malnutrition on a much smaller geographical scale. More work is needed to better target future campaigns, particularly those aimed at diseases with heterogeneous distributions, such as urinary schistosomiasis and malaria.

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HEALTH SERVICES FOR BURULI ULCER CONTROL: LESSONS FROM A FIELD STUDY IN GHANA

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Buruli ulcer (BU), caused by *Mycobacterium ulcerans* infection, is a debilitating disease of the skin and underlying tissue. The first phase of a BU prevention and treatment programme (BUPaT) was initiated from 2005-2008, in the Ga-West and Ga-South municipalities in Ghana to increase access to BU treatment and to improve early case detection and case management. This paper assesses achievements of the BUPaT programme and lessons learnt. It also considers the impact of the programme on broader interests of the health system. A mixed methods approach included patients' records review, review of programme reports, a stakeholder forum, key informant interviews, focus group discussions, clinic visits and observations. Extensive collaboration existed across all levels, (national, municipality, and community), thus strengthening the health system. The programme enhanced capacities of all stakeholders in various aspects of health services delivery and demonstrated the

importance of health education and community-based surveillance to create awareness and encourage early treatment. A patient database was also created using recommended World Health Organisation (WHO) forms which showed that 297 patients were treated from 2005-2008. The proportion of patients requiring only antibiotic treatment, introduced in the course of the programme, was highest in the last year (35.4% in the first, 23.5% in the second and 42.5% in the third year). Early antibiotic treatment prevented recurrences which was consistent with programme aims. In conclusion, to improve early case management of BU, strengthening existing clinics to increase access to antibiotic therapy is critical. Intensifying health education and surveillance would ultimately increase early reporting and treatment for all cases. Further research is needed to explain the role of environmental factors for BU contagion. Programme strategies reported in our study: collaboration among stakeholders, health education, community surveillance and regular antibiotic treatment can be adopted for any BU-endemic area in Ghana.

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REPORT ON THE MARCH 2011 MCGILL-PAHO WORKSHOP ON DEWORMING OF PRESCHOOL CHILDREN IN THE AMERICAS

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Preschool children are one of three population groups at highest risk for soil-transmitted helminth infections. Because deworming coverage in this group is sub-optimal, new strategies for engagement of governments and other partners are needed. With support from the Canadian Institutes of Health Research, a workshop co-sponsored by McGill University and PAHO was held in March 2011 to review the situation on deworming of preschool children in the Americas. A total of 33 participants represented a variety of organizations, including WHO, the Global Network for Neglected Tropical Diseases, NGOs (including faith-based organizations), academia and national governments. Lessons learned from two long-standing and successful deworming programs, those of Mexico and Nicaragua, were highlighted and served as the basis for a discussion of current challenges experienced in other countries. The workshop recommendations addressed issues of political commitment, integration of deworming with other child health programs, national action plans incorporating NTDs, intersectoral coordination and partnerships, advocacy, capacity-strengthening, community participation and social mobilization, innovation in communication strategies, diagnostic tools, drug formulations and other tools, development and dissemination of guidelines among UN agencies and other organizations, research gaps, South-South collaboration, development of reporting systems, optimal delivery strategies, setting coverage goals and scaling-up activities within the PAHO 10-year plan for Comprehensive Child Health. It is expected that the momentum generated by this workshop, in addition to massive recent donations of deworming drugs, will accelerate national action plans and inform WHO's new Strategic Plan for the Control of Soil-transmitted Helminths for the next decade.

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BIOTECHNOLOGY COMPANIES AND EMERGING MARKET DEVELOPERS PARTICIPATE SIGNIFICANTLY IN R&D FOR NEGLECTED DISEASES

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Understanding the full spectrum of organizations participating in research and development (R&D) for neglected diseases is essential to inform the design of new programs and initiatives to fill gaps in the neglected disease R&D pipeline. In 2011, BIO Ventures for Global Health (BVGH) published an expanded edition of its Global Health Primer, a tool that compiles, tracks, and analyzes the pipeline for drugs, vaccines, and diagnostics in development for neglected diseases. Further analysis of drugs and vaccines in development for 17 neglected diseases was performed using the Global Health Primer dataset and identified 313 products in development by 288 distinct organizations. Organizations identified included academic/research institutions, government agencies, product development partnerships (PDPs), biotechnology companies, and pharmaceutical companies from 42 countries. Two groups of developers identified in this analysis are of particular interest. First, biotechnology companies currently participate in the development of 120 drugs or vaccines for 14 neglected diseases, accounting for 27% of the number of distinct organizations participating in product development. Beyond neglected diseases, the biotechnology sector has played an increasingly important role in the development of innovative solutions to human health challenges. Therefore, increasing participation by this sector represents a key opportunity to increase innovation in the neglected disease R&D pipeline. Second, developers from emerging market countries, focused here on Brazil, China, India, and South Africa, are participating in the development of 42 drugs or vaccines for 13 neglected diseases and represent 15% of the distinct organizations participating in product development. As the economies of emerging market countries grow, they are likely to increase their capacity for scientific research, novel product development, and inexpensive product manufacturing, thus increasing their potential to contribute to neglected disease R&D pipelines. The data presented here will inform future efforts to promote partnering, policy, and financial support mechanisms to engage product developers and address unmet needs in the neglected disease R&D pipeline.

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POST-PREVENTIVE CHEMOTHERAPY COVERAGE SURVEY IN SIERRA LEONE: NATIONAL VALIDATION OF REPORTED DRUG DISTRIBUTION COVERAGE DATA FOR NEGLECTED TROPICAL DISEASE CONTROL

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From October 2008 through February 2009, ivermectin and albendazole were distributed to 3.2 million Sierra Leoneans in 13 districts as preventive chemotherapy (PCT) for lymphatic filariasis, onchocerciasis, and soil transmitted helminthes. Although supervision by national program staff during PCT was routinely conducted, a post-PCT coverage validation survey was conducted in April 2009 so that reported coverage rates could be validated, detailed information could be collected on gender- and age-specific coverage by district, reasons why people chose not to take the drug could be compiled, and the current strategies assessed. At the national level and in eight districts, reported coverage rates fell outside of the surveyed coverage confidence intervals for ivermectin and/or albendazole. The largest differences between the two coverage rates were in the districts of Kono and Moyamba, the two districts with the greatest variation in population changes due to post-war migration, with increases that are not reflected in the national census projections. In the Rural Western Area health district, the large confidence intervals suggested a need to validate coverage through alternative means. Gender- and age- specific survey data were assessed: in ten districts, there were no significant differences in coverage between males and females, while females were significantly less likely to be treated compared to males in three districts ($p < 0.05$). Of the age categories examined, those 15-29 years old were the least likely to take both drugs compared to ages 5-14 and those greater than 30 years. The most common reasons

given for not receiving treatment were being underage, absent at the time of distribution, and that the drug distributor did not visit the house. Recommendations based on survey results will be used in subsequent years to strengthen the PCT strategies through increased field supervision, heightened monitoring and evaluation, improved drug availability, more robust social mobilization campaigns, and an improved method to validate coverage data.

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BREAKING INTELLECTUAL PROPERTY (IP) BARRIERS TO ACCELERATE DRUG RESEARCH AND DEVELOPMENT (R&D) FOR NEGLECTED TROPICAL DISEASES

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The Pool for Open Innovation against Neglected Tropical Diseases ("the Pool"), administered by BIO Ventures for Global Health, motivates innovative and efficient drug discovery and development by opening access to intellectual property (IP) or know-how in neglected tropical disease (NTD) research. Intellectual property concerns have been at the heart of access to medicines. Intellectual property includes patents_which give the owner a period of time to exclusively market a new product, and know-how the accumulated experience that companies gain over time through working on new drugs. The Pool makes thousands of patents and associated know-how accessible to qualified researchers working on research and development (R&D) on drugs for NTDs, allowing these researchers to take advantage of hundreds of millions of dollars of value accumulated in the IP of companies and universities. The project engages novel mechanisms, such as profiling specific patents with potential application to new drug discovery and development for NTDs, to make the patents more accessible to researchers and highlight new project opportunities focused on drug discovery and development. For example, GlaxoSmithKline (GSK) has contributed a family of patents covering small molecules with antibacterial activity. GSK has biological data relevant to tuberculosis and malaria for compounds based on this chemical scaffold that can serve as a starting point for follow on work in tuberculosis and malaria or novel projects for leprosy and Buruli ulcer. Ultimately, by opening access to their IP, contributing organizations offer an opportunity to gain invaluable 'know-how' that can advance existing research efforts. This poster will provide a brief overview of the project structure and core principles, as well as demonstrate examples of how researchers can engage with the Pool to accelerate the pace of R&D for NTDs.

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AZITHROMYCIN DISTRIBUTION USING COMMUNITY VOLUNTEERS: COSTS OF DISTRIBUTION IN SEVEN DISTRICTS IN PLATEAU AND NASARAWA STATES, NIGERIA

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Mass treatment with azithromycin is a key component of the WHO SAFE strategy to eliminate blinding trachoma. In Nigeria, the first ever mass administration of donated azithromycin to combat blinding trachoma (donated by Pfizer for this purpose) took place in 2010. As an alternative strategy to current trachoma control program practice, MDA in Plateau and Nasarawa states was carried out by community volunteers, with support from the Ministry of Health and The Carter Center. A total of 769,517 treatments in 7 local government areas (districts) of either azithromycin tablets, pediatric oral suspension, or ophthalmic tetracycline ointment were distributed representing an estimated 77.7% coverage of the total population. The Ministry of Health provided personnel but no direct costs; since The Carter Center provided all direct funding, all costs could be monitored through financial records. Costs were assessed according to input (per diem, transport, and materials and supplies) as well as activity (advocacy, training, distribution, and supervision). A total of $47,243 was spent, $20,762 (43.9%) of which was used for clearing and shipping of the drug. The remaining $26,481 accounted for all distribution costs. Personnel costs (per diems) was the largest input at $14,684 (55.5%) while training of MOH supervisors and community volunteers accounted for greatest activity cost at $10,704 (40.4%). The total and mean cost per treatment was $0.04 (range $0.02 to $0.05), not including clearing and shipping costs. Not included in this analysis were salaries, overhead costs for The Carter Center or Ministry of Health, or drug costs (except tetracycline eye ointment). Some economies of scale were seen in larger local government areas where per person treatment costs reduced compared to smaller local governments.

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DETERMINATION OF THE SENSITIVITY AND SPECIFICITY OF THREE SERODIAGNOSTIC ASSAYS FOR CHAGAS DISEASE BY LATENT CLASS ANALYSIS

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Chagas disease, caused by the hemoflagellate *Trypanosoma cruzi*, is an increasing public health concern in the Unites States because of the estimated 300,000 infected residents, the majority of who came to the country from endemic areas. During the extended chronic stage of this disease, the parasitemia is very low and difficult to detect, therefore serological assays assume primacy for diagnosis. There is no gold standard for the serodiagnosis of Chagas disease, thus the WHO recommends that diagnosis is based on the concordant results of 2 different serologic tests. In the absence of a gold standard, diagnosis of disease is imperfect. We used Latent Class Analysis (LCA) to determine the sensitivity and specificity of 3 serodiagnostic assays for Chagas disease. LCA statistically models the results from the different tests to the underlying latent classes (positive or negative). The analysis yields probabilities for each combination of results from which the sensitivity and specificity of the each assay can be estimated. To generate the data for LCA, we tested a serum bank with 3 assays: the Trypomastigote Excreted Secreted Antigen Immunoblot (TESA IB), the CDC Chagas immunofluorescence assay (IFA) and the commercial Chagatest *ELISA recombinante v.3.0* (Wiener Laboratorios, Argentina). The serum bank (n = 605) comprised sera submitted to CDC for routine diagnosis of Chagas disease (n = 479), plus 126 true negative specificity controls that were positive for diseases which can cause cross reactivity in Chagas serology and came from areas where Chagas disease is absent or transmission is very rare. We performed LCA on the entire set (n = 605). All 3 assays returned sensitivity and specificity values >94%. We also estimated the specificity of each assay by standard 2 x 2 tables using data from the true negatives (n = 126). These specificity results were: TESA IB 99.2%, IFA 93.7%, Chagatest ELISA 86.5%. These data provide an independent estimate of the performance of these assays and support their use for serodiagnosis.

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IN THE SEARCH FOR MARKERS OF CHEMO-RESISTANCE IN AMERICAN TEGUMENTARY LEISHMANIASIS (ATL)

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Therapeutic failure in leishmaniasis is a common problem in endemic areas. This may occur due to altered drug pharmacokinetics, re-infection, or immunologic compromise of the host. However, in many cases it may be partly due to parasite drug resistance. No reliable markers of chemo-resistance against leishmanicidal drugs have been described until yet, and the only reliable method for monitoring resistance of individual isolates is the *in vitro* amastigote-macrophage model, as reported

previously. It is thus necessary to uncover cellular and molecular indicators to be used systematically to identify the drug-resistant phenotype of the infecting parasites. Herein we analyze in parasites isolated from three patients suffering ATL and lack of response to antimonials their capacity to accumulate calcein, the rate of glucose uptake and the membrane potential, and compared the results with those obtained from references strains belonging to *Leishmania. braziliensis L. mexicana* and *L. amazonensis*. Our results suggest that some of the isolates a) have an increased expression of ABC transporters; b) accumulate glucose at a lower rate and c) have a less polarized membrane potential compared to the reference parasites. Additionally they suggest that some of these isolates express different sensitivity of the membrane potential to classic inhibitors of the mitochondrial function. Altogether these results indicate that in parasites isolated from ATL patients suffering chemotherapeutic failure there could be physiological changes that might serve as markers of chemo-resistance and be helpful for designing strategies to circumvent *Leishmania* drug-resistance and successfully treating leishmaniasis. If this conclusion holds true particularly in isolates obtained from patients, its prognostic value treatment outcome might be extremely useful.

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ANALOGUES OF FENARIMOL AS NOVEL COMPOUNDS FOR THE TREATMENT OF CHAGAS DISEASE

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A collaborative drug discovery consortium established by not-for-profit, drug research and development organization Drugs for Neglected Diseases *initiative* (DND*i*) has identified and developed novel compound series active against intracellular protozoan parasite *Trypanosoma cruzi* the causative agent of Chagas disease. Compounds are derived from the fungicide fenarimol reported to inhibit ergosterol biosynthesis by binding to fungi CYP 51. Fenarimol derivatives are able to suppress bloodstream parasitemia to virtually undetectable levels after once-a-day oral dosing in a mouse model of chronic *T. cruzi* infection. Compounds are non-cytotoxic and chemically tractable allowing rapid optimization of target biological activity and drug characteristics. Chemical and biological studies undertaken in the development of this series of compounds towards the goal of delivering new drug candidates for Chagas Disease will be presented.

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A MEMBER OF RAS ONCOGENE FAMILY, RAP1A SIGNALING, MEDIATES ANTILEISHMANIAL ACTIVITY OF MONASTROL

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Microarray experiments were conducted on Affymetrix GeneChip® HG-U133 Plus 2.0 array to determine the genes that encode proteins related to pathological alterations of cell signaling pathways in intracellular *Leishmania* amastigotes in response to the oral antileishmanial agent, monastrol. Monastrol, the investigational compound, with antileishmanial activity targeting pteridine reductase (PTR1) in *Leishmania* parasites, induced unprenylated Rap1A when exposed to this anticancer drug at IC_{50} of 10 μM. Monastrol is known to cause mitotic arrest in cancer cells, inhibited Rap1A prenylation (geranylgeranylation) in intracellular *Leishmania* which results in blockade at the G1 phase of the cell cycle. Regulators (unprenylation) of cell signaling pathways can be exploited in *Leishmania* parasites as novel therapeutic tools. We propose the development of antiparastic drugs to 'piggyback' on the development of inhibitors for cancer research targeting farnesyltransferase and geranylgeranyltransferase.

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MOLECULAR DIAGNOSIS OF LEISHMANIASIS AT THE COMPLEX AND SPECIES LEVEL IS IMPORTANT FOR CLINICAL MANAGEMENT

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The intracellular parasite *Leishmania* causes a wide spectrum of human disease, ranging from self-healing cutaneous leshimaniasis to fatal visceral leishmaniasis. *Leishmania* is a digenetic obligate intracellular protozoan parasite. Management depends on the clinical syndrome which is a function of the species complex. Drug resistance has also been associated with certain species. Culture or stain-based methods do not distinguish complex or species of *Leishmania*. We report a real-time PCR (RT-PCR) assay for laboratory diagnosis of Leishmaniasis by the detection of *Leishmania* complexes (L. Viannia, *L. mexicana*, *L. donovani/infantum, L. major, L. tropica*) directly from clinical samples. To highlight the utility of molecular detection and complex idenfictcation two clinical cases of cutaneous leishmaniasis are presented. Case 1 was a 31 year-old-man born in Syria who immigrated to Canada one year prior to presentation. He had an 18 month history of numerous cutaneous lesions on his forearms bilaterally which could be interpreted as disseminated disease consistent with a more virulent species. The possibility of visceral disease prompted a more aggressive approach to management in this individual with sodium antimony gluconate 1900 mg IV daily for 20 days. The RT-PCR performed on skin scraping of the lesion from this case interestingly identified species *L. tropica*. Case 2 was a 49 year-old-man born in Canada. He developed a papule on his right arm which later ulcerated four months after he traveled to Surinam for one month. The patient did not receive treatment. The cutaneous leishmaniasis lesion healed spontaneously with some postinflammatory hyperpigmentation. The RT-PCR performed on skin scraping of the lesion from this case however identified *L. (Viannia) panamensis* (Braziliensis complex). We conclude that *Leishmania* complex or species identification is useful in the management of this disease.

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DRUG DISCOVERY ALGORITHM FOR CUTANEOUS LEISHMANIASIS

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The results of our automated, high-throughput screening of potential drugs *in vitro* against promastigotes was recently published and the complimentary exercise against axenic amastigotes will be detailed elsewhere. Because no drug has been specifically developed for cutaneous leishmaniasis, there are no examples of a pre-clinical product evaluation scheme that leads to agents for formal non-clinical and clinical development. We have developed a testing strategy that features a gated, resource sparing model that progresses from high-throughput *in vitro* promastigote and axenic amastigote assays to more clinically relevant, comparable mouse *Leishmania* suppression and *Leishmania* cure models that have undergone internal validation and are reproducible. Our process for advancing compounds from hit to lead will be discussed.

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OPTIMIZING ANALOGS OF TIPIFARNIB AS *TRYPANOSOMA CRUZI* CYP51 INHIBITORS

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Tipifarnib is an oral anti-cancer drug clinical candidate that blocks mammalian protein farnesyltransferase (PFT). In previous work, we reported that tipifarnib is a potent inhibitor of *Trypanosoma cruzi* growth, but acts via inhibition of the parasite's sterol 14α-demethylase (CYP51) enzyme. Since the antitrypanosomal activity is unrelated to inhibition of PFT, new tipifarnib analogs were designed to minimize PFT inhibition and to maximize binding to the *T. cruzi* CYP51. The purpose was to eliminate side effects, such as bone marrow suppression, that are associated with PFT inhibition. The design of analogs was aided by the crystal structures of both mammalian PFT and the *T. cruzi* CYP51. Compared to the parent compound (tipifarnib), the analogs have as much as 10,000-fold higher IC_{50} values on mammalian PFT and >10-fold lower EC_{50} values on *T. cruzi* cultures (in the sub-nanomolar range). The compounds rank amongst the most potent anti-*T. cruzi* compounds that have ever been discovered. The compounds also have potent activity in the mouse model of *T. cruzi* infection, and new *in vivo* data will be presented. By starting with the tipifarnib scaffold, we hope to retain some advantageous pharmacological properties, namely, oral bioavailability with excellent human pharmacokinetics, low inhibitory activity on liver P450 enzymes, and a simple chemical structure with low cost of goods. These intrinsic attributes may have advantages over antifungal CYP51 inhibitors that are also under investigation for Chagas disease.

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ISOLATION OF NOVEL STEROLS WITH ANTI-LEISHMANIAL ACTIVITY FROM THE MAYAN PLANT *PENTALINON ANDRIEUXII* MUELLER-ARGOBIENSIS

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Pentalinon andrieuxii has been used by traditional Mayan healers for topical treatment cutaneous leishmaniasis in the Yucatan Peninsula. Chemical analysis of this plant revealed the presence of a new cholesterol derivative, that we named pentalinonsterol, and a new polyoxygenated pregnane sterol glycoside that we named as pentalinonoside, we also isolated 18 known compounds, that includes 14 sterols, three coumarins, and a triterpene. All these compounds were isolated from an *n*-hexane partition of a methanol extract of the roots of the plant. Structure elucidation of all 20 compounds was accomplished by spectroscopic procedures. Experiments performed *in vitro* revealed that 6 out of those 20 compounds present a strong antileishmanial activity against promastigotes of *Leishmania mexicana* as detected *in vitro* by flow cytometry. Additionally, sterols were active against intracellular amastigotes grown in mouse macrophages. We conclud that six sterols from *P. andrieuxii* present anti-leishmanial activity *in vitro* and as such could be leads for developing new drugs.

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IDENTIFICATION OF ANTILEISHMANIAL BENZOTHIAZOLES BASED ON HITS FROM A HIGH-THROUGHPUT PROMASTIGOTE SCREEN

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A screen of ~200,000 compounds from the PubChem database revealed 93 compounds that possessed IC_{50} values ≤ 1 μM against *L. major* promastigotes, as reported previously. To identify new compounds active against intracellular *Leishmania*, thirty-four of these compounds were selected according to chemical exclusion criteria and availability, purchased from commercial suppliers, and evaluated for *in vitro* activity against intracellular *L. donovani* and *L. amazonensis* parasites. Benzothiazole compounds (PubChem 16196319 and 16196223) related to cyanine dyes exhibited potent activity against intracellular amastigotes, leading to a search for structurally related and commercially available compounds. The cyanine dye thiazole orange (Pubchem 123859) showed exceptional *in vitro* antileishmanial activity, particularly against intracellular *L. donovani* (IC_{50} = 21 ± 12 nM) and low cytotoxicity against Vero cells (IC_{50} = 7800 ± 231 nM). Dithiocarbamates also showed nanomolar *in vitro* antileishmanial activity, as the aldehyde dehydrogenase inhibitor disulfiram possessed *in vitro* potency (IC_{50} = 43 ± 6 nM) which was similar to the reference drug amphotericin B. Several of the most potent compounds have been evaluated for their efficacy in a murine model of visceral leishmaniasis. Thus far, the most promising compounds from *in vivo* studies have been benzothiazoles 123859 and 16196319. When given at a dose of 1 mg/kg i.p. daily for five days, 123859 and 16196319 cause 44% and 42% suppression of liver parasitemia in *L. donovani*-infected BALB/c mice, respectively, compared to the untreated control group. Benzothiazole-containing cyanine dyes are thus potential lead compounds for the discovery of novel antileishmanial agents.

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OPTIMIZATION OF CONGENITAL CHAGAS DISEASE TREATMENT WITH BENZNIDAZOLE

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The main difficulty in the treatment of congenital Chagas disease regards compliance to the treatment: over 40% of treatments are stopped before achievement. We conducted a clinical trial to compare two doses of benznidazole to see whether the simplification and reduction of treatment could induce a better compliance. The study was conducted in 3 hospitals in the city of Santa Cruz in Bolivia. Newborns of all seropositive women for *Trypanosoma* cruzi were investigated for *T. cruzi*. We confirmed the cure for all children by parasitological examination at 1 and 2 months and by serological surveillance using Chagas Stat-Pak®, confirmed by an ELISA using recombinant antigens (Chagatest®, Wiener, Argentina) performed at 1, 2 and 9 months. The comparison of compliance between the two groups was based on both the use of electronic pillboxes (MEMS®, AARDEX, Switzerland) registering each opening of the bottle, and a weekly visit at home to check treatment attendance. We compared the total dose taken with the prescribed dose. Infected newborns were randomly divided into 2 groups: 64 infants received treatment A (5 mg/kg per day in 2 divided doses for 60 days) and 61 infants were treated by treatment B (7.5 mg/kg per day in 1 single dose for 30 days). Taking into account refusals, abandonments and deaths, the numbers of followed infants were respectively 59 and 54 infants. There was no significant difference (P> 0.05) between the groups A and B regarding a) the average number of days without treatment, b) the frequency of days without

treatment, c) the number and importance of treatment-free periods exceeding 3 consecutive days and d) the prescribed dose and the dose taken. Our results showed that compliance was not significantly improved by streamlining the processing or shortening. However, simplification of treatment (once daily instead of two) could allow, at a time, to reduce the doses and maintain the same efficiency.

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IDENTIFICATION OF SERUM BIOMARKERS FOR CHAGAS DISEASE

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The blood borne pathogen *Trypanosoma cruzi*, is the etiological agent of Chagas disease in humans. Following a natural infection some individuals exhibit an acute phase, with parasites present in blood, while 20 to 30% of individuals develop chronic Chagas disease with clinical symptoms starting many years after the initial infection. Most chronically infected individuals show no clinical symptoms and may donate blood, resulting in an increased risk of transfusion transmitted Chagas disease. Parasites are rarely detected in blood in chronically infected individuals. To overcome the difficulty of detecting parasites directly, diagnostic assays detect host anti-*T. cruzi* antibodies as a surrogate marker for infection. However, these assays are not reliable during the initial window period, or to follow cure after drug treatment due to the persistence of parasite specific antibodies. Previous studies have determined that the parasites secrete various antigens in the blood and these have been collectively termed as *T. cruzi* Excreted Secreted Antigens (TESA). We utilized *in-vitro* RNA SELEX methods to develop TESA aptamers (short nucleic acid molecules) with the goal of utilizing them as specific ligands in detection assays. The TESA SELEX was performed using culture supernatants of *T. cruzi* trypomastigote infected NIH-3T3 cells. Biotinylated monoclonal TESA specific aptamers were utilized in a modified enzyme linked assay to detect TESA antigens in *T. cruzi* infected mouse plasma. Aptamer L44 (AptL44) demonstrated a consistent and strong binding to its target in infected mouse plasma during the acute phase. This interaction was specific as a scrambled aptamer did not bind to either infected or uninfected mouse plasma. AptL44 was also able to detect its target in plasma from chronically infected mice, 135 days post infection, where no parasites were detectable in blood by microscopy. Further analysis of the binding properties of AptL44 and the identification and purification of its target are being carried out. This is the first demonstration of an aptamer based assay that detects a parasite biomarker for the diagnosis of Chagas disease.

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DRUG SUSCEPTIBILITY OF *LEISHMANIA* VIANNIA SPECIES IN COLOMBIA

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Treatment failure is frequent and concerning in the management of cutaneous leishmaniasis in South America. Treatment with Glucantime® has been shown to select antimony (Sb^v)-tolerant/resistant parasites and drug resistance to contribute to treatment failure. To determine the susceptibility of *Leishmania* affecting human populations in Colombia to currently used anti-leishmanial drugs, we evaluated *in vitro* susceptibility of 150 clinical strains of *L. braziliensis, L. panamensis* and *L. guyanensis* from endemic regions to Glucantime® and miltefosine. Susceptibility was determined based on reduction of intracellular parasite burden in U-937 macrophages by screening at single drug concentrations and ED50 determination. Sb and miltefosine resistant lines and their wild type strains provided internal standards. Susceptibility to miltefosine and Glucantime® differed among species and by geographic origin. Low susceptibility was defined as < 50% reduction of parasite burden at the screening concentration of 32mgSb^v/ml (based on C_{max} of Sb in plasma) and 16uM for miltefosine (based on toxicity of higher concentrations for U-937 cells). 20-50% of *L. panamensis* and 40-53% *L. braziliensis* strains presented low susceptibility for Sb^v; 14-80% of *L. panamensis* and 58-79% for *L. braziliensis* presented low susceptibility to miltefosine. All *L. guyanensis* strains were highly susceptible to both Sb^v and miltefosine. *Leishmania* from the Orinoco and Amazon River regions were less sensitive to both drugs than strains from other high transmission areas. *L. braziliensis* presented low sensitivity to both drugs more frequently than other *(Viannia)* species. No significant difference in susceptibility to Sb was detected among strain cohorts (N=85) isolated between 1980-1989 and 2000-2009 in the municipality of Tumaco. However a higher proportion of strains from the Rosario river focus presented low susceptibility than strains from the Mira river focus (50% vs 27%, *p=0.032*). These results support both intrinsic and acquired differences in drug susceptibility of *L. (Viannia)* species.

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CYP51 INHIBITORS IN CLINICAL TRIALS FOR THE ETIOLOGICAL TREATMENT OF CHAGAS DISEASE

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Chagas disease, caused by the kinetoplastid protozoon *Trypanosoma cruzi*, remains the highest parasitic disease burden in the American continent and is now spreading to non-endemic countries due to international migrations. Specific chemotherapy of this complex and long-neglected disease remains unsatisfactory due to limited efficacy of currently available drugs (nifurtimox, a 5-nitrofuran and benznidazole, a 2-nitroimidazole), particularly in the prevalent chronic stage, as well as unwanted side effects that can lead to treatment discontinuation. Currently, the most advanced candidates for new specific treatments are a group of third-generation triazole derivatives, originally developed for the treatment of invasive fungal infections, which are potent and selective inhibitors of fungal and protozoal cytochrome P-450-dependent C14α sterol demethylase (CYP51). These compounds have been shown to induce radical parasitological cures in different animal models of acute and chronic Chagas disease, being active *in vivo* against nitrofuran- and nitroimidazole-resistant *T. cruzi* strains, even if the hosts are immunosuppressed. The remarkable *in vivo* antiparasitic activities of these CYP51 inhibitors result from a combination of their potent and selective intrinsic anti-*T. cruzi* activity with special pharmacokinetic. Among this group of compounds, posaconazole (Noxafil®, Merck) and ravuconazole (Eisai), both of which have completed preclinical studies, are currently entering clinical trials for the etiological treatment of chronic Chagas disease. A Phase II clinical trial of the comparative efficacy and safety of posaconazole and benznidazole in chronic Chagas disease patients was started in Vall d'Hebron University Hospital, Barcelona, Spain in October 2010. Also in 2010, Merck announced plans to initiate a Phase II investigational proof-of-concept clinical study to evaluate posaconazole for the treatment of chronic Chagas disease in Argentina and Brazil, with an estimated start date of 2Q 2011. On the other hand, the Drugs for Neglected Diseases initiative (DNDi) announced in 2009 that it had reached an agreement with Eisai for the clinical development of E1224, a water-soluble pro-drug (mono-lysine derivative) of ravuconazole, for the treatment of chronic human Chagas disease in Bolivia; the estimated start date is April 2011.

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INTERACTION MAP OF LYT1 FROM *TRYPANOSOMA CRUZI*

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Trypanosoma cruzi is the etiologic agent of Chagas' disease. This parasite requires infecting the host cell to complete its intracellular cycles, a process in which the involvement of very few molecules of the parasite has been described. LYT1 is a lytic molecule active in acid conditions that, by genetic approach, we demonstrated its participation in the parasite infection and stage-transition processes. This multi- functionality are the result of the production of two LYT1 products obtained by alternative trans-splicing in which the full-length LYT1 protein contains an amino-terminal signal sequence and an internal sequence which directs nuclear localization, whereas the truncated protein lacks the secretion sequence. Therefore, one form of the LYT1 protein is secreted and participates in hemolysis, infectivity and the parasitophorous vacuole escape. The other form is located in the kinetoflagellar and nucleus zone and is involved in the parasite developmental process. This dual/single-gene expression and consequent differential localization and functional switching of protein products, expose these molecules to different microenvironments that could impact on protein folding and interaction with other proteins. Therefore, in this work we performed co-immunoprecipitation and GST pull-down assays followed by MS-MS analysis, to obtain the LYT1 interactome. The co-immunoprecipitation assays demonstrated than LYT1p interacts with at least 14 proteins from 8 to 255 kDa range of molecular weight, which interact with different forces according with crescent salt stringency experiments. In the same way, by GST pull-down assays we observed an interaction with at least 8 proteins from 27 to 100 kDa range of molecular weight, which also showed different interaction forces. The MS-MS analysis allows us to identify proteins that are related with the infection or stage-transition process of *T. cruzi*, in which the participation of LYT1p has been demonstrated.

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DIFFERENTIAL GENE EXPRESSION IN DEVELOPMENT OF *TRYPANOSOMA BRUCEI* DURING SALIVARY GLAND COLONIZATION OF *GLOSSINA MORSITANS MORSITANS*

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African trypanosomes (*Trypanosoma brucei* sspp) are the etiological agent of the fatal human disease, Sleeping Sickness, in sub-Saharan Africa. This parasite is transmitted to the vertebrate host by the bite an infected tsetse fly (*Glossina* spp.). When the fly feeds on an infected host, trypanosomes differentiate to the procyclic form in the midgut (MG). Parasites migrate to the proventriculus (PV) and then to salivary glands (SG), where epimastigotes attach to the epithelium, and differentiate ultimately becoming the mammalian-infective metacyclics. SG invasion occurs in only a subset of flies that carry midgut infections. In non-permissive flies, the infection is halted in the PV. The molecular aspects that govern SG invasion are currently unknown. In this work, from an Illumina transcriptome data set, we have analyzed the differential regulation of four putative proteins sharing type II phosphatidic acid phosphatase (PAPs) motifs. In eukaryotic cells, PAP activity has a central role in phospholipids and triacylglycerol synthesis through its product diacylglycerol, and also generates and/or degrades lipid-signalling molecules related to phosphatidate. Three of the four proteins are predicted to be transmembrane proteins that cross the lipid bilayer six times. Structural homology comparison suggests that the catalytic site of these enzymes is exposed to the extracellular mileu. cDNAs from infected tissues were normalized to trypanosome alpha-tubulin using RT-PCR. Normalized cDNAs were tested with gene specific primers for the four genes. Further, PV cDNAs (prepared as above) from both SG permissive and non-permissive flies were similarly analyzed. Three of four genes were found to be highly expressed only in infected SG. Genes expressed highly in the SG were also found to be expressed at a higher level in the PV from SG permissive flies. We hypothesize these enzymes could be involved in cell signaling processes regulating SG invasion by *T. brucei* and/ or *T. brucei* differentiation in permissive flies. Characterization of these proteins could increase our understanding of SG invasion processes by *T. brucei*.

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DIFFERENCES IN THE *IN SITU* INFLAMMATORY REACTION OF THE AMERICAN TEGUMENTARY LEISHMANIASIS AND SPOROTRICHOSIS AS AN EXAMPLE OF THE SKIN IMMUNE SYSTEM RESPONSE PATTERNS

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The skin is an important immune surveillance organ that is target by many infectious agents. The clinical presentation of skin infectious diseases can be influenced by the interaction between the skin immune system (SIS) and intracellular or extracellular pathogens, such as *Leishmania* spp and Sporothrix schenckii, respectively. As concequence, American tegumentary leishmaniasis (ATL) and sporotrichosis (SP) could be influenced by the pathogen-skin immune system (SIS) interaction. To better clarify the underlying mechanisms of skin inflammation in the presence of different pathogens, we used immunohistochemistry to analyze 3 groups of patients with lymphocutaneous (LC) and fixed (F) forms of sporotrichosis and cutaneous form of ATL. ATL lesions had a significantly higher percentage of $CD3^+$ cells than LC ($p= 0.012$) and F ($p= 0.009$), $CD8^+$ cells ($p= 0.001$ and $p= 0.002$, respectively), macrophages ($p= 0.003$ and $p= 0.025$), $FasL^+$ cells ($p= 0.001$ and $p= 0.003$) and NOS2 ($p= 0.007$ and $p= 0.0001$). In contrast, LC lesions had a significantly higher percentage of dendritic cells ($p= 0.026$), neutrophils ($p= 0.009$) and $CD22^+$ cells ($p= 0.024$), than ATL lesions. The clinical presentation of ATL and sporotrichosis could be due to a combination of factors from the host SIS and etiological agent. In addition, the results also indicated a different profile of the *in situ* immune response when ATL, LC-SP and F-SP are compared, suggesting that the SIS is a complex, adaptable system that is capable of different responses to intracellular or extracellular pathogens.

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DRUG LEADS TARGETING *TRYPANOSOMA CRUZI* CYP51 IDENTIFIED BY HIGH-THROUGHPUT SMALL MOLECULE SCREENING

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Chagas Disease, a WHO- and NIH-designated neglected tropical disease, is endemic in Latin America and an emerging infection in the US and Spain as a result of population movements. Although a major cause of morbidity and mortality due to heart failure, as well as exacting a heavy economic toll in affected regions, Chagas Disease attracts little attention from the pharmaceutical industry because of adverse economic incentives. Discovery and development of new routes to chemotherapy for Chagas Disease is a clear priority. To maintain membrane integrity, *Trypanosoma cruzi*, the etiological agent of Chagas Disease requires endogenously synthesized episterol and fecosterol, membrane building blocks that cannot be entirely substituted by cholesterol scavenged from the host. The similarity between the sterol requirements of pathogenic fungi and *T. cruzi* validated the strategy of repurposing anti-fungal drugs inhibitors of CYP51 to treat Chagas Disease. To supply the therapeutic pipeline with novel

anti-Chagasic drug candidates, we exploited a complementary approach that relies on directly probing the *T. cruzi* CYP51 active site with synthetic small molecules to select chemotypes with high binding affinities to the target. Our approach incorporates screening technologies against both the target enzyme and the parasites, x-ray crystallography and computation. This strategy allows the unique binding features of positive hits to be elucidated, with the ultimate goal of utilizing them in the subsequent hit-to-lead optimization. This approach has enabled us to identify novel scaffolds which show significant diversification from previously identified anti-fungal drugs. One potent *T. cruzi* inhibitor with drug-like properties is currently being optimized.

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GENE EXPRESSION PROFILES OF HUMAN MACROPHAGES INFECTED WITH *LEISHMANIA BRAZILIENSIS IN VITRO*

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The protozoan parasite *Leishmania braziliensis* has a high degree of intra-species genetic and phenotypic polymorphism, which is accompanied by a spectrum of clinical presentations in the infected human host, including: localized cutaneous leishmaniasis (CL), mucosal leishmaniasis (ML) and the more recently described disseminated leishmaniasis (DL). Our hypotheses are (1) that these parasites interfere with the gene expression of infected cells in a manner that is beneficial to their infectivity, and (2) that strains of *L. braziliensis* drawn from patients with either CL, ML or DL lead to different gene expression profiles in the infected macrophages. Employing DNA micro-array we compared the global gene expression profiles in human monocyte derived macrophages (MDM), obtained from healthy donors and infected in parallel with one *L. braziliensis* isolated from a CL, one from a ML and one from a DL case of the same endemic region in Northeastern Brazil. We also assessed how infected MDM compared with non-infected cells. Overall, *L. brazilienis* caused the repression of the majority of the genes that presented significant changes of their expression levels in infected MDM as compared to non-infected cells. In this respect, genes belonging to the stimulus transmission, apoptosis and reactive oxygen production pathways were the most affected. Interestingly, genes for proteins involved in stress protection were up-regulated. Among the three isolates tested, the two drawn from metastatic disease cases (i.e. ML and DL) induced more similar gene expression patterns in the MDM. The findings suggest that these parasites may increase their chance of survival by down regulating host cell genes during the infection process, and that strains associated with different forms of disease elicit somewhat diverse behaviors in host cells, which may be related to the different clinical outcomes of the disease.

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THE COMPLEX ROLE OF PROGRAMMED DEATH-1 (PD-1) IN CHRONIC ZOONOTIC CANINE VISCERAL LEISHMANIASIS

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The ability of the immune system to effectively respond to *Leishmania infantum* infection, the causative agent of visceral leishmaniasis (VL), is dependent upon a classic Th1 response including antigen-specific proliferation of CD4+ T cells. Functional exhaustion of T lymphocytes has not previously been identified during VL. Treatment of clinical VL is complicated by an attenuated immune response at the time of therapy, resulting in incomplete parasite clearance and possible recrudescence. Zoonotic canine VL serves as a pertinent and useful model to better understand cellular mechanisms which alter clinical disease. Our previously published research suggested the presence of CD4+ T-cell exhaustion in poly-symptomatic dogs chronically infected with *L. infantum,* as evidenced by their significantly reduced $CD4^+$ T cell proliferation in response to specific antigen and significant production of IL-10 after *ex vivo* antigen stimulation. Our hypothesis was that peripheral blood mononuclear cells (PBMC) from dogs diagnosed with *L. infantum* via serology and qRT-PCR would have increased expression of the surface receptor PD-1. PBMC from dogs in three different symptomatic states were evaluated for responsiveness to *L. infantum* antigen, surface expression of PD-1, and production of IL-10 and IFN-γ ex vivo. CD4+ T cells from dogs with immune exhaustion had significantly elevated PD-1 expression compared to naive and infected immune-responsive dogs. Dogs with elevated PD-1 and poly-symptomatic clinical disease demonstrated decreased IFNγ producing CD4+ cells and increased IL-10 producing CD4+ T cells. These novel findings suggest a complex role for PD-1 in regulation of the chronic immune response to *L. infantum*. Recovery of a productive and efficacious CD4+ T lymphocyte response in VL would improve the efficacy of current therapeutic options and reduce the duration of treatment necessary to achieve remission of clinical disease and parasitological cure.

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CONTROL OF PARASITE GROWTH VERSUS PATHOLOGY IN *LEISHMANIA BRAZILIENSIS* INFECTION

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Cutaneous leishmaniasis (CL) and mucosal leishamaniasis (ML) are characterized by high production of pro-inflammatory cytokines and development of pathology. In contrast, 70% of the individuals exposed to *Leishmania braziliensis* infection do not develop disease. Sub-clinical *L. braziliensis* infection (SC) is characterized by a positive delayed type hypersensitivity test (DTH) and production of lower amount of IFN-γ and TNF-α than CL and ML patients. Here we evaluate why individuals with SC *L. braziliensis* infection had a weak type 1 immune response and how they control leishmania infection. Cytokines (IL-10 and IL-23) and neutralizing antibodies anti cytokine were added to lymphocyte cultures. The ability of macrophages from individuals with different clinical forms of *L. braziliensis* infection to produce chemokines and kill *L. braziliensis* was determined. Cytokines and chemokines were measured in lymphocyte and macrophage cultures by ELISA and PCR. The production of IL-10 and IL-27 were not enhanced in SC and neutralization of IL-10 did not enhance IFN-γ production in these individuals. While macrophages from CL and ML patients produced higher amounts of CXCL9, CXCL10 and TNF-α than SC subjects, killing of *L. braziliensis* was higher in SC individuals than in CL and ML patients. These data show that macrophages and lymphocytes from CL and ML patients produce higher levels of pro-inflammatory chemokines and cytokines that were associated with pathology. In contrast, macrophages from SC individuals kill more efficiently *L. braziliensis* than macrophages from CL and ML, indicating that protection is associated with the innate immune response.

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GROWTH KINETICS AND CELL VIABILITY OF FIVE REFERENCE STRAINS OF *LEISHMANIA* FROM THE WORLD HEALTH ORGANIZATION

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Leishmaniasis is a public health problem in countries with endemic zones. Since 1972 the reference strains of the WHO have been used in *Leishmania* research. Once these strains are acquired, they are cultivated and amplified without knowing relevant information, like growth kinetics, which could affect the diagnosis of the disease and the associated research. The aim of this study was to characterize the growth kinetics and cell viability of five reference strains of *Leishmania* obtained from the WHO and to determine changes in growth kinetics between the evaluated

species and along passages. The growth kinetics was performed in the passages 1, 5 and 10 by determining promastigote daily concentration and viability, using propidium iodide staining and the software CellProfiler until a zero viability was obtained. The evaluated species were: *Leishmania braziliensis* (MHOM/BR/75/M2903), *L. panamensis* (MHOM/PA/71/LS94), *L. guyanensis* (MHOM/GF/79/LEM85), *L. amazonensis* (MHOM/BR/73/M2269) y *L. mexicana* (MHOM/BZ/82 BEL 21). The results showed that the species of the subgenus *Leishmania* reached the maximum growth between the third and fourth day of culture, while species of the subgenus *Viannia* did it at sixth and seventh day of culture. Differences between passages 5 and 10 were not found among almost all growth kinetic curves. However, after comparing these two similar passages with passage 1 it was found that the final concentration of parasites at the end of their logarithmic phase was twofold higher in passage 1 for *L. amazonensis* and a half fold for *L. guyanensis* and *L. mexicana*. For the other species there were not differences between passages. Once the logarithmic phase had come to its end, the parasites viability decreased from 100% to values near to 0%. In conclusion, we found differences in the growth kinetics and viability of the parasites between the subgenus *Leishmania* and *Viannia* but not among the species of each subgenus. We found differences in the growth kinetics between the passages, but patterns were different for each species.

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CPG (TLR9) MEDIATED IMMUNOTHERAPY OF CHRONIC *LEISHMANIA (VIANNIA) PANAMENSIS* INFECTION IN THE MOUSE MODEL

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Leishmania (Viannia) are the primary agents of cutaneous leishmaniasis in the Americas. New therapeutic approaches are necessary, as current treatment strategies are hindered by severe side effects, lengthy treatment regimens and emerging drug resistance. Patients infected with L. (V.) panamensis display a mixed Th1/Th2 cytokine profile, which is replicated in a chronic infection BALB/c mouse model. Utilizing this model, the therapeutic potential of the TLR9 agonist, unmethylated CpG, which is known to promote a Th1 cytokine response, was evaluated. Mice, with established lesions, treated with CpG, had significantly reduced lesions compared to controls. Surprisingly, when the draining lymph node response was analyzed directly after treatment, there was significantly reduced IFNγ, IL-10, IL-13 and IL-17 in treated mice. Further, an increased IL-10:IFNγ ratio was observed. Consistent with these observations, *in vitro* experiments revealed that draining lymph node cells from infected mice, when treated with high (1uM) doses of CpG in the presence of soluble leishmanial antigen, produced reduced IFNγ and increased IL-10 responses. These results suggested that down-regulation of immune and inflammatory responses were involved in disease amelioration. To test this, infected mice were treated with the indoleamine 2,3-dioxygenase (IDO) inhibitor, 1-methyl tryptophan (1-MT), which is known to inhibit regulatory T cell development. Mice treated with 1-MT, developed larger lesions, higher parasite burdens, and increased cytokine production of IFNγ, IL-10, IL-13 and IL-17. These results are consistent with studies utilizing CpG treatment in autoimmune disease and suggest that CpG may act by inducing a beneficial Treg response that dampens lesion site cellular recruitment and pathology of leishmaniasis. Further studies are in progress to further evaluate the CpG regulatory response to determine if this may provide an alternative approach to current chemotherapeutic treatment.

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FREQUENCY OF LYSOSOME DEPENDENT AND INDEPENDENT CELL ENTRY BY THE TCI LINEAGE OF *TRYPANOSOMA CRUZI*

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Chagas disease is caused by *Trypanosoma cruzi*, an obligate intracellular protozoan parasite. An essential part of *T. cruzi's* life cycle is the process of cell invasion, which is required for parasite multiplication inside its mammalian host. *T. cruzi* is capable of invading non-phagocytic host cells through two different mechanisms. In the "lysosome-dependent pathway" (LDP), the parasite enters the host cell surrounded by vacuoles derived from host cell lysosomes. In contrast, in the "lysosome-independent pathway" (LIP), *T. cruzi* enters the host cell enveloped in a plasma membrane-derived vacuole, which only later acquires lysosomal markers. Recent studies based on *in vitro* assays show that the LIP is more frequently used by Y-strain (TcII lineage) parasites. However, *T. cruzi* is a highly heterogeneous species, whose six distinct lineages (TcI-TcVI) differ widely in their biological properties. At present, information about frequency of use of each cell invasion mechanism by other *T. cruzi* lineages is not available. We measured the infectivity and the frequency of usage of each invasion mechanism by *T. cruzi* parasites belonging to the TcI lineage (three different isolates and the Brazilian strain). Although we found large differences among isolates regarding *in vivo* and *in vitro* infectivity, we determined that the frequency of usage of each of the two invasion routes is very similar to those of the TcII lineage (Y-strain). Our results suggest that the frequency with which each cell invasion mechanisms is used by different isolates is independent of their degree of infectivity. In addition, they suggest that preference for the LIP invasion route is independent from the parasite lineage.

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*LMEX*NUC-1 AND *LMEX*NUC-2: TWO FUNCTIONALLY IMPORTANT SECRETORY/RELEASED NUCLEASES OF *LEISHMANIA MEXICANA*

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Leishmaniasis affects 2 million people worldwide; its symptoms range from localized cutaneous lesions to systemic disease. *Leishmania* promastigotes (Pro) are transmitted to mammals via the bite of an infected sand fly. In the mammalian host Pro transform into amastigotes (Am) which reside and multiply within macrophage phago-lysosomal vacuoles. All *Leishmania* species are purine auxotrophs; i.e. they need to acquire these essential molecules from their mammalian/insect hosts. Thus it is relevant to investigate the purine scavenging pathways and enzymes of this unicellular parasite. Using molecular biology techniques we demonstrated that *L. mexicana* release/secrete two 35kDa nucleases-nucleotidases: *Lmex*NUC^{s}-1 and -2. The two enzymes are located on chromosome 29 and are 95% identical. RT-PCR and Northern-blot analyses showed that expression of *Lmex*NUC^{s}-1 and -2 is higher in Am compared to Pro. Over-expression experiments and confocal microscopy showed that both, *Lmex*NUC^{s}-1 and -2 are synthesized by amastigotes while inside macrophage phago-lysosomal vacuoles. The tertiary protein structure of *Lmex*NUC^{s}-1 and -2 chimeras including several disulfide bonds and a metal co-factor (Zn^{2+}) were found essential for enzyme function. Anti-sense experiments suggested that *Lmex*NUC^{s}-1 and -2 were not absolutely essential for parasite survival *in vitro* using single polynucleotide purine substrates. However, such anti-sense transfectants showed that *Lmex*NUC^{s}-1 and -2 are necessary for parasite invasion/survival in J774 mouse macrophages. Our cumulative results suggest that *Lmex*NUC^{s}-1

and -2 play important role(s) in facilitating the growth, development and survival of this important human pathogen. Thus, these enzymes may represent logical targets for therapeutic intervention,

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FUNCTIONAL CHARACTERIZATION OF A GENE ENCODING A UNIQUE DEVELOPMENTALLY EXPRESSED SECRETORY INVERTASE FROM *LEISHMANIA MEXICANA*

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Leishmania parasites are all transmitted by phlebotomine sand flies. Within these vectors, these parasites multiply and move anteriorly in the sandfly alimentary tract. During this migration these parasites must obtain host derived nutrients/energy sources to survive and multiply. Sand flies characteristically ingest plant sugars, including sucrose and other polysaccharides, and store these sugars in their crop. Between blood meal feeds they regurgitate such sugars into their anterior mid-gut. In that regard, recently, we found that *L. mexicana* promastigotes (Pro) secrete/release an invertase/sucrase activity into the culture medium during growth *in vitro*. In contrast, *L. mexicana* axenic amastigotes (AxAm) do not release any detectable invertase activity. To characterize this invertase activity further, we adopted a molecular approach. Using PCR methods, we identified a gene which encodes a putative *L. mex* invertase (LmxM04.0310; *LmxINV*). Results of RT-PCR demonstrated that mRNA for LmxINV was expressed only by *L. mex* Pro and was not detected in *L. mex* AxAm. The *LmxINV* encodes a 71.5 kDa protein with conserved β-fructofuranosidase domains and a secretion signal peptide. To characterize this enzyme further, we designed an expression constructs containing a C-terminal hemagglutinin tag (*LmxINV:HA*). This was ligated into the leishmanial episomal expression vector pKSNEO. Following electroportation, *L. mex* transfectants were selected for growth in increasing concentrations of G418. Results of enzyme assays demonstrated that such transfectants expressed more than 100 fold higher levels of secreted invertase activity than vector-match controls. Such activity was readily immuno-precipitated with anti-HA monoclonal antibody beads. Western blots of such immuno-precipitates showed only a single ~72 kDa band of LmxINV:HA protein. Such transfectants were readily propagated as promastigotes but were incapable of true transformation into axenic amastigotes *in vitro*. These results suggest that LmxINV has additional roles in developmental biology of this human pathogen.

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HISTOPATOLOGICAL CHANGES IN CARDIAC TISSUE FROM *CAVIA PORCELLUS* EXPERIMENTALLY INFECTED WITH *TRYPANOSOMA CRUZI*

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We have previously shown that guinea pigs infected with *Trypanosoma cruzi* develop histopathological changes with similar characteristics to the human Chagas disease. Our aim was determine whether variations in levels of collagen I, III and IV are related to the parasite load and the degree of inflamation in the cardiac tissue, during the experimental infection of guinea pigs by *T. cruzi*. Seventy-two guinea pigs were inoculated intradermally with 10^4 trypomastigotes of *T. cruzi* strain Y (experimental group, EG), and 18 guinea pigs were used as a control group (CG). Eight animals from EG and two from CG were sacrificed at 5, 15, 20, 25, 40, 55, 115, 165 and 365 days post infection. The immunotypes of collagen (CI, CIII and CIV) were detected by immunohistochemistry. We observed a decrease in levels of collagen I during the acute phase (20-55 days pi) and it was associated with high numbers of amastigote nests and severe inflammation. During the early chronic phase (115-165 days pi) there was a slight increase in the levels of the three types of collagen examined. An increase in levels of CI, CIII and CIV was observed during the chronic phase (365 days pi), where CIII and CIV had the highest levels. Also in this phase, CI and CIII were detected in interstitial and perivascular spaces and CIV was detected in some interstitial forms and on the basement membrane of cardiomyocytes. The deposits of collagen were associated with inflammatory cells such as lymphocytes and some macrophages, suggesting an association of the inflammatory process with fibrogenesis in chronic Chagas disease. Additionally, during that phase, the fibrosis in cardiac tissue was associated with the presence of parasite DNA. These results show the usefulness of the guinea pig as an animal model to explain cardiac remodeling during *T. cruzi* infection.

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PATHOGENICITY OF *PLASMODIUM FALCIPARUM* FIELD ISOLATES AND INHIBITION OF HUMAN ENDOTHELIAL CELL APOPTOSIS BY FASUDIL

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Plasmodium falciparum infection can abruptly progress to severe malaria, a life-threatening complication resulting from sequestration of parasitized red blood cells (PRBC) in the microvasculature of various organs such as the brain and lungs. PRBC adhesion can induce endothelial cell (EC) activation and apoptosis, thereby disrupting the blood-brain barrier. Moreover, the hemozoin, the malarial pigment induces the erythroid precursor apoptosis. Despite the current efficiency of antimalarial drugs in killing parasite, severe malaria still causes up to one million deaths every year. A new strategy targeting both parasite elimination and EC protection is urgently needed in the field. Recently, a rho-kinase inhibitior Fasudil, a drug already in clinical use in human for cardio- and neuro-vascular diseases, was successfully tested on laboratory strains of *P. falciparum* to protect and to reverse damages of the endothelium. We therefore assessed herein whether Fasudil, would have a similar efficiency on *P. falciparum* taken directly from malaria patients using contact and non contact experiments. Seven (23.3%) of 30 PRBC preparations from different patients were apoptogenic, four (13.3%) acting by cytoadherence and three (10%) via soluble factors. None of the apoptogenic PRBC preparations used both mechanisms indicating a possible mutually exclusion of signal transduction ligand. Three PRBC preparations (42.9%) induced EC apoptosis by cytoadherence after 4 h of coculture ("rapid transducers"), and four (57.1%) after a minimum of 24 h ("slow transducers"). The intensity of apoptosis increased with time. Interestingly, Fasudil inhibited EC apoptosis mediated both by cell-cell contact and by soluble factors but did not affect PRBC cytoadherence. Fasudil was found able to prevent endothelium apoptosis from all the *P. falciparum* isolates tested. Our data provide evidence of a strong anti-apoptogenic effect of Fasudil and show that endothelial cell-*P. falciparum* interactions are more complicated than previously thought. These findings may warrant clinical trials of Fasudil in severe malaria management.

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CALCIUM AND ERYTHROCYTE INVASION DURING SICKLE CELL ANEMIA

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Regulation of intracellular Ca^{2+} is essential for *Plasmodium falciparum* development and survival and invasion of the red blood cell (RBCs) can induce senescence. In the same line increase of the Ca^{2+}level in RBCs can trigger eryptosis. In RBCs with sickle cell trait (HbSS) Ca^{2+} concentration is thus considerably higher than in normal ones (HbAA) and is associated with premature senescence and eryptosis. This can limit the lifespan of the parasite in patients with sickle cell anaemia and may explain their relative protection against malaria. However recent studies warn that children with sickle cell anaemia are more likely to die from severe malaria, suggesting that parasites can survive within this hostile environment. To deal with this topic, we use homozygote HbSS RBCs to study invasion and development of *P. falciparum* during sickle cell anaemia. Using FLUO4-AM we compare the calcium content of HbSS and HbAA infected RBC. Over 72h of culture, we found that parasite grow slower in HbSS RBCs than in HbAA ones with a delay in the life cycle. Using Q-PCR we found no change in PfATP6, PfATP4, PfV1, PfV2, PfCAX, PfNHE expression between the two set of parasites. By flow cytometry we found a great heterogeneity of Ca^{2+} level in HbSS RBCs, with 21.2% harbouring a high concentration of Ca^{2+}. Double staining of parasitized erythrocytes with Fluo4-AM and hydroethidine showed no significant difference in the Ca^{2+} content of HbAA and HbSS parasitized RBCs (Fluo4 mean of fluorescence 3.33 and 4.40 respectively). These results draw question about whether parasites infect only HbSS RBC with a normal intracellular Ca^{2+} level or whether they regulate the Ca^{2+} content of the RBC early after invasion without evidence of modulation of the main cation exchanger expression.

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AN ENDOGENOUS NITRIC OXIDE SYNTHASE INHIBITOR IS ASSOCIATED WITH SEVERE MALARIA IN GAMBIAN CHILDREN

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Low nitric oxide (NO) bioavailability contributes to systemic endothelial dysfunction in severe malaria. NO generation by nitric oxide synthase (NOS) requires arginine as a substrate and is inhibited by asymmetric dimethylarginine (ADMA). The ratio of arginine to ADMA determines NO production by NOS. We hypothesized that the ratio of arginine to ADMA in blood plasma would be decreased in Gambian children with severe malaria. We enrolled children with malaria at community health centers near Fajara, The Gambia. We classified patients as mild, moderately severe (prostration), or severe (coma, respiratory distress or severe anemia) using clinical criteria. Arginine and ADMA were measured at admission and 28 days after discharge. We studied 102 mild, 45 moderately severe, and 51 severe malaria patients. The plasma arginine concentration was decreased among patients with either moderately severe or severe malaria compared to patients with mild malaria (Mild: 45.0 [IQR 35.4 - 55.7], Moderate: 25.5 [IQR 20.4 - 39.2], Severe: 32.8 [IQR 25.6 - 40.7] µmol/L, $p \leq 0.001$). The plasma ADMA concentration was elevated specifically in patients with severe malaria (0.44 [IQR 0.37 - 0.60] µmol/L) compared to patients with mild malaria (0.40 [IQR 0.33 - 0.47] µmol/L, $p = 0.02$) or moderately severe malaria (0.34 [IQR 0.27 - 0.43] µmol/L, $p = 0.002$). Plasma arginine was positively correlated with plasma ADMA; therefore, we analyzed the ratio of Arginine to ADMA. Arg:ADMA was 113.5 (IQR 86.3 - 138.2) in children with mild malaria, 82.9 (IQR 68.2 - 93.7) in children with moderately severe malaria, and 62.9 (IQR 52.9 - 79.3) in children with severe malaria ($p \leq 0.001$ for each comparison). 28 days after discharge, the Arg:ADMA ratio improved substantially within each group to 174.6 (IQR 148.0 - 209.5, $p < 0.0001$) among children with mild malaria, 144.0 (IQR 100.4 - 176.7, $p < 0.0001$) among children with moderately severe malaria, and 125.1 (IQR 99.6 - 153.3, $p < 0.0001$) among children with severe malaria. Significant differences in the Arg:ADMA ratio persisted between children recovered from mild malaria and children recovered from either moderate or severe malaria ($p < 0.005$). Severe malaria in Gambian children is associated with an acute decrease in the ratio of Arginine to ADMA, a change that could diminish endothelial cell nitric oxide synthesis. Therapeutics that inhibit ADMA release or increase ADMA clearance may help to restore endothelial function in children with severe malaria.

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TOWARDS UNDERSTANDING THE PATHOPHYSIOLOGY OF RETINOPATHY NEGATIVE CEREBRAL MALARIA BY COMPARING RATES AND TYPES OF VIRAL COINFECTIONS IN MALAWIAN CHILDREN WITH OR WITHOUT MALARIA PARASITEMIA

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The underlying pathophysiology of retinopathy negative cerebral malaria (CM) is unknown. Determining the rates and types of viral (co)infectors in patient with and without malarial parasitemia may help eludicte the role of viral (co)infection in the pathophysiology of this condition. We analyzed blood and CSF samples in three groups to compare rates and types of viral (co)infectors: retinopathy negative CM; retinopathy positive CM; and children in coma without circulating malaria parasites. The rates of viral (co)infection differed among the three studied groups. These findings lend support to the hypothesis that viral coinfection may be important in the pathophysiology of retinopathy negative CM. Our laboratory analyses had several limitations and future studies may better characterize rates and types of viral (co)infections in these three groups. Expansion of the numbers of patients sampled may further improve understanding of the pathophysiology of retinopathy negative CM.

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MALARIA PATHOGENESIS: MICROFLUIDIC MODELING OF SPLENIC FILTRATION IN MALARIA PATIENTS AT A CLINICAL FIELD SITE

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Splenic filtration is hypothesized to contribute to the pathogenesis of complicated malaria infections. We developed a method for estimating splenic filtration during malaria infection based on characterizing red blood cell (RBC) populations in peripheral circulation. Using microfluidic devices, we measured the surface area and volume of thousands of individual red blood cells from each patient. The minimum cylindrical diameter can be calculated from the surface area and volume. This parameter describes the smallest tube or pore that a cell can traverse without lysing. The minimum cylindrical diameter is useful in describing the probability of a cell becoming filtered by the spleen. This idea centers on the concept that cell geometry and not the dynamics of deformation are important to filtration of RBCs in the spleen. If the spleen is constantly filtering RBCs by their minimum cylindrical diameter, then the dimensions

of the circulating RBC population can describe splenic filtration. By measuring thousands of parasitized and normal RBCs in an individual, we can derive a model to estimate splenic filtration of parasitized RBCs in that individual. We applied this modeling technique at a field site in Blantyre, Malawi to determine if there are innate differences in an individual's RBCs or splenic filtration that could affect the presentation of malaria infection. We quantified differences between parasitized RBCs observed in peripheral circulation to those grown in culture. We observed samples from 120 individuals classified into 4 groups: cerebral malaria, uncomplicated malaria, aparasitemic coma, and healthy controls. We were able to see statistically significant differences in the uninfected RBCs from healthy controls and malaria patients. We did not see differences in the estimated splenic filtration rates between cerebral malaria and uncomplicated malaria patients. This is the first field-study where statistically significant sizes of patient populations have been analyzed with microfluidic devices to understand physiological variations between living individuals.

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AN N-ETHYL-N-NITROSOUREA (ENU)-INDUCED MUTATION IN JAK3 PROTECTS AGAINST CEREBRAL MALARIA BUT CAUSES SUSCEPTIBILITY TO MYCOBACTERIA

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Cerebral malaria (CM) is an acute, often lethal neurological complication of malaria. The cell and molecular pathways involved in CM pathogenesis are poorly characterized and need to be better understood to identify novel therapeutic targets for intervention. CM can be modeled in mice by infection with *Plasmodium berghei* ANKA. To identify genes and proteins involved in CM pathogenesis and whose inhibition may be of clinical value, we set up a forward genetic screen in ENU-mutagenized F2 mice to identify recessive mutations that protect mice against *P. berghei*-induced CM. We identified a pedigree (P48) segregating a resistance trait (in 31% of progeny) whose protective effect was fully penetrant on C57BL/6J and 129S1 genetic backgrounds, and that was mapped to the central portion of chromosome 8. Whole genome sequencing of CM-resistant P48 animals identified homozygosity for a missense mutation (W81R) in the Band 4.1/Ezrin/Radixin/Moesin (FERM) domain of the Janus-associated kinase 3 (Jak3) protein. The causative effect of W81R was verified by complementation testing, with $Jak3^{-/W81R}$ double heterozygotes being fully protected against *P. berghei*-induced CM. Immunological characterization of $Jak3^{W81R}$ homozygotes showed defects in thymic development, with concomitant severe depletion of $CD8^+$ T cells, B cells and NK cells. There was also defective T cell-dependent production of IFN- upon mitogen stimulation. Adoptive transfer of infected splenocytes from *P. berghei* infected C57BL/10J mice abrogated CM resistance in $Jak3^{W81R}$ homozygotes, an effect largely attributed to the $CD8^+$ T cell compartment. Paradoxically, $Jak3^{W81R}$ homozygotes were highly susceptible to mycobacterial infections with *Mycobacterium bovis* (BCG), *M. tuberculosis* and *Citrobacter rodentium* whose resolution depends on a robust Th1 immune response. These findings highlight the pathological role of $CD8^+$ T cell and IFN-γ-dependent Th1 responses in CM pathogenesis. They identify a direct role for Jak3 in this process, and suggest possible novel strategies for intervention in CM.

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SURVIVAL OUTCOME AND IMMUNOLOGICAL MECHANISMS OF CO-INFECTION OF SCHISTOSOMIASIS AND MALARIA IN A PRIMATE MODEL

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Multiple infections are a common phenomenon in developing countries especially Africa. Human studies have established that this is particularly true for malaria and schistosomiasis, since the epidemiological distribution of both diseases overlap. In order to determine the effect of multiple infections on disease outcome, we conducted a controlled experiment to investigate the effect of chronic schistosomiasis on severe malaria (SM) and acquired immunity to malaria infection in the baboon. To understand the underlying mechanisms, we analyzed immunological parameters associated with SM during co-infection and determined how worm infection affects development of acquired immunity to malaria. The experiment was conducted in two phases; effect of chronic schistosomiasis on severe malaria (phase 1) and acquired immunity (phase 2). In phase 1, four groups of baboons were used. Groups A, B and C were infected with 500 *Schistosoma mansoni* cercarie. To determine the effect of treatment on co-infection, group A was treated with praziquantel at week 14 and 15. Four weeks later, groups A, B and D were inoculated with 105 *Plasmodium knowlesi* parasites. In phase 2, three groups of baboons were used. Groups A and B were subjected to the same procedure as B and C above. However after schistosome treatment all groups were infected twice with *P. knowlesi*, cured with artemether/lumefantrine at 2% parasitaemia and challenged a third time. Results from phase 1 showed that *P. knowlesi* infected animals had an early onset of parasitemia and succumbed to SM unlike majority of co-infected baboons. For animals with both infections, we noted elevated levels of Th2 cytokines (especially IL-6) and T-regulatory markers prior to malaria infection. In phase 2, all animals were protected from SM after multiple infection and treatment. This study demonstrates that chronic schistosomiasis reduces SM and this could be mediated by pro-inflammatory cytokines and suggests a role for T-regulatory pathways. On the other hand, the presence of schistosomiasis does not affect acquired immunity to malaria infection in baboons.

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THE IMMUNOMODULATORY ACTIVITY OF MALARIA PIGMENT (HEMOZOIN) ON THE INNATE IMMUNE RESPONSE: PLATELETS AND MONOCYTES AS KEY EFFECTOR CELLS

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Malaria has profound worldwide impacts, infecting millions of people annually with the highest mortality rates occurring in the children of sub-Saharan Africa infected with *Plasmodium falciparum*. The parasite's life cycle occurs within infected erythrocytes and produces hemozoin, a crystalline metabolite of hemoglobin digestion, which is released during malaria infection and found in high concentrations in the circulation. The effects of the key malarial toxin on human platelet response have not been examined. Therefore, we characterized the interaction of pure, synthetic hemozin (sHz) on human platelets and monocytes *in vitro*. We observed that surface P-selectin and PAC-1 binding were increased when human platelets were stimulated of sHz (2-20μM) and that sHz (2μM) potentiated platelets activation by thrombin (0.01 μ/mL). In addition, sHz also induced release of platelet factor 4 and RANTES by human platelets. Futhermore, sHz increased golgi apparatus compared to thrombin activation based on transmission electron microscopy and immunocytochemsitry, suggesting that sHz alters post-translational processing of proteins in platelets. We also found that sHz triggers platelet aggregates and formation of heterotypic aggregates with human monocytes, a sensitive marker of

platelet activation. Platelet-monocyte aggregates formed in whole blood and in isolated cell suspension in response to sHz. This was interrupted by a blocking antibody against P-selectin indicating that binding P-selectin to P-selectin glycoprotein ligand 1 (PSGL-1) on the monocyte which mediates both adhesion and signaling is a key mechanism. Our observations demonstrated that sHz is an agonist for platelet activation and interactions with monocytes, that it may have important roles in platelet-mediated events in clinical malarial syndromes.

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We also found that sHz triggers platelet aggregates and formation of heterotypic aggregates with human monocytes, a sensitive marker of platelet activation. Platelet-monocyte aggregates formed in whole blood and in isolated cell suspension in response to sHz. This was interrupted by a blocking antibody against P-selectin indicating that binding P-selectin to P-selectin glycoprotein ligand 1 (PSGL-1) on the monocyte which mediates both adhesion and signaling is a key mechanism. Our observations demonstrated that sHz is an agonist for platelet activation and interactions with monocytes, that it may have important roles in platelet-mediated events in clinical malarial syndromes.

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DELETION OF ADß2 INTEGRIN PROTECTS AGAINST EXPERIMENTAL CEREBRAL MALARIA

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Malaria, a disease of major importance in many areas of the world, causes a variety of pathologic syndromes including cerebral malaria, which is often fatal. Leukocyte integrins are essential for host defense but also mediate pathologic as well as physiologic responses of the innate and adaptive immune systems. Their roles in malarial syndromes have not been defined. We previously showed that targeted deletion of the αD subunit (αD-/-) of αDβ2 integrin, which is expressed on key macrophage subsets in mice and humans, leads to absent expression of the integrin heterodimer on murine leukocytes and reduces mortality of mice infected with *Plasmodium berghi* Anka (PbA), a cause of experimental cerebral malaria. To further indentify mechanisms that are involved, we examined wild type (WT) and αD-/- mice at 7 and 10 days after PbA infection and found significant decreases in vessel plugging, micro-hemorrhages, and necrosis in the brains of αD-/- animals. Intravital microscopy and flow cytometry demonstrated lower numbers of rolling and adherent leukocytes in cerebral vessels of αD-/- mice, and decreased T lymphocyte accumulation in the brains of infected animals. Evans blue dye exclusion assays demonstrated significantly less dye extravasation in the brains of αD-/- mice at day 10, indicating preserved blood-brain barrier integrity. Furthermore, there were altered patterns of inflammatory cytokine expression in αD-/- compared to WT mice at 7 and 10 days. Neurophysiologic analysis indicated that αD-/- mice had improved cognitive function, which may result from reductions in brain vasculopathy, leukocyte sequestration, and inflammation. We conclude that deletion of αDβ2 alters the natural history of severe experimental malaria, demonstrating previously-unrecognized activities of a key leukocyte integrin in immune and inflammatory responses in this syndrome.

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CHARACTERIZATION OF A NOVEL FAMILY OF LONG NON-CODING RNA TELOMERE-ASSOCIATED REPETITIVE ELEMENT (LNCRNA-TARE) TRANSCRIPTS IN *PLASMODIUM FALCIPARUM*

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The importance of long non-coding RNAs (lncRNAs) in epigenetic remodeling and transcriptional regulation has been recently established across numerous eukaryotic systems. Given *Plasmodium falciparum*'s susceptibility to histone deacetylase inhibitors and mounting evidence for epigenetic regulation of multi-gene virulence families, we hypothesized that lncRNAs are involved in the *P. falciparum* transcriptional network. Using a high-resolution DNA tiling microarray, we have identified and characterized several putative *P. falciparum* lncRNAs, including a novel family of twenty-two long non-coding RNA telomere-associated repetitive element (lncRNA-TARE) transcripts. LncRNA-TARE transcripts are coordinately expressed after parasite DNA replication from at least eighteen chromosome termini and encompass the majority of known binding sites (SPE2) for the ApiAP2 transcription factor PfSip2. Interestingly, the SPE2 binding site is only otherwise found in the promoter of *upsB*-type *var* genes, and an *upsB*-type *var* gene is adjacent to each lncRNA-TARE gene. LncRNA-TARE is thus poised to play an important role at *P. falciparum* chromosome ends.

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ELEVATED SERUM HEME OXYGENASE-1 LEVELS ARE ASSOCIATED WITH NEUROLOGIC PROTECTION IN CHILDREN WITH CEREBRAL MALARIA

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Heme oxygenase-1 (HO-1) degrades heme into biliverdin, carbon monoxide and free ferrous iron and acts as a regulator of pro- and anti-inflammatory cytokine activity. HO-1 is associated with protection from development of experimental cerebral malaria in murine models. HO-1 protection to malaria is thought to be due to effects against inflammation and oxidative stress. To further investigate the role of systemic HO-1 in cerebral malaria (CM) pathogenesis, we have measured serum HO-1 levels in Ugandan children with CM (n=74), uncomplicated malaria (UM, n=68), and healthy community children (CC, n=60). HO-1 levels (median [interquartile range], ng/mL) were higher in Ugandan children with CM (28.4 [61.4]) than in children with either UM (16.5 [54.3], *P*=0.05) or

community children (8.3 [8.3], *P*<0.0001). Children with CM who did not have neurologic deficits at discharge or at 3-month follow-up had higher admission serum HO-1 levels than those who did have deficits at those time points (discharge: 36.1 [83.0] vs 23.4 [14.5], *P*=0.07; 3-month follow-up: 28.7 [61.6] vs 14.4 [2.2], *P*=0.03). Elevated serum HO-1 levels were not associated with protection from death. HO-1 levels are elevated in children with CM and are associated with neuroprotection in these children.

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UMBILICAL AND UTERINE ARTERY DOPPLER STUDIES AMONG MALARIA INFECTED AND NOT INFECTED PREGNANT WOMEN

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Malaria complicated pregnancies are a significant public health problem affecting mothers and their offspring. In this longitudinal cohort study, our goal was to determine if abnormalities *in utero*placental blood flow were indicative of a malaria complicated pregnancy. Pregnant women were recruited from Msambweni Kenya, at the time of their first antenatal visit. Patients with known medical disorders contributing to fetal growth restriction, placental dysfunction, and prematurity were excluded. Using a SonoSite 180 Plus ultrasound machine, the uterine and umbilical artery Doppler indices were studied in addition to fetal biometrics. Malaria infection was determined by PCR from maternal blood samples taken at the time of the first clinic visit and at delivery (maternal venous, placental-intervillous, and cord blood). Newborn birth weight, length, and head circumference were measured. Study outcomes were stratified by 3 week gestational age groups and compared in malaria infected vs. not infected women. 471 women were enrolled. Malaria prevalence for study participants was ~7%. In 18-23 week gestational age groups, women with malaria infections had increased umbilical artery pulse index (PI), resistance index (RI) and systolic/diastolic (S/D) ratios compared to women not infected with malaria. This effect was not seen in later gestational age groups. No difference in uterine artery Doppler indices was found between malaria infected and not infected women. These umbilical artery abnormalities were not observed in later periods of pregnancy. Doppler measured abnormalities may be indicative of malaria complicated pregnancies. Given the small sample size, further research is needed. Malaria prophylaxis should be encouraged in all pregnant women as early as possible in pregnancy.

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ORIGIN OF A LINAGE OF *PLASMODIUM* SPECIES IN ORANGUTAN

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The large number of *Plasmodium* samples recently obtained from African Apes has provided new perspectives on the evolution of human and ape malarias. A missing piece of the puzzle, however, are the malarias found in Apes from Southeast Asia. In this study, we report molecular data for a malaria parasite lineage found in orangutans. Twenty four blood samples were collected in 2003 at a "Orangutan Care Center and Quarantine (OCC&Q)" in Indonesia. We screened the samples for *Plasmodium* parasites by nested PCR using the cytochrome b (cyt b) gene. For all positive samples, parasite mitochondrial genomes (mtDNA) and two antigens: circumsporozoite protein gene (CSP) and merozoite surface protein 1 42kDa (MSP-142), were amplified, cloned, and sequenced. We found 15 orangutans positive by PCR using cytb primers. These isolates yielded five distinctive mitochondrial haplotypes not previously found in non-human primates and were found to exhibit low genetic divergence among them suggesting that they belong to one species. Whereas positive blood smears were available, we could not establish whether they belong to any of the two previously described orangutan malarias. We report a phylogenetic analysis that includes this parasite from orangutan using complete mitochondrial genomes, CSP and MSP-142 separately. Our phylogenetic analyzes revealed that the orangutan malaria lineage was part of a monophyletic group that includes all the known non-human primate malarias found in Southeast Asia; specifically, it shares a recent common ancestor with *P. inui* (a macaque parasite) and *P. hylobati* (a gibbon parasite). This finding suggests that this lineage may have originated as a result of a host switch from a non-Ape host. As has been previously observed in the other *Plasmodium* species found in non-human primates, the CSP protein shows high polymorphism in the number of repeats. The polymorphism found in the non-repetitive 3' and 5' regions is similar to the one reported for other parasites and appears to be neutral. In contrast, the genetic diversity of MSP-142 in orangutan is twice that observed in *P. vivax* and seems to be under positive selection. This result is similar to previous findings in non-human primate malarias closely related to *P. vivax*. The limited molecular evidence available from Asian Apes indicate that these parasites originated independently from those found in Africa, likely as the result of host switches from other non-human primates.

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IN THE PERUVIAN AMAZON, MALARIA INFECTION SEVERITY IS ASSOCIATED WITH HOST AND PARASITE VARIABLES AND GROWTH *IN VITRO*

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It is widely reported that not all *Plasmodium falciparum* isolates are capable of adapting to culture. We looked for variables that predict *in vitro* growth considering 1663 *P. falciparum* infections of which 274 were put directly into culture. Parasite growth was measured by *ex-vivo* parasite multiplication rate (ex-PMR, fold increase in parasitemia between generations) and overall culture success over 21 days. Variability in parasite genotype was investigated for associations with disease parameters and *in-vitro* growth using 14 microsatellite markers and polymorphisms in the Merozoite Surface Protien-1 block 2 (MSP1-B2). We considered *in vitro* growth dynamics using a novel quantitative PCR method, which was validated by comparing 80 clinical with nested PCR and capillary gel electrophoresis techniques. Parasite density was associated with presence or absence of fever, complexity of infection, microsatellite cluster, ex-vivo PMR and growth success ($p<0.0001$ for all associations). There was an inverse correlation between starting in-vitro parasitemia and ex-PMR during the first 48 hours (low parasitemia PMR=1.7, high parasitemia PMR= 2.5; $p=0.0002$). We suspect this ex-PMR difference is related to host immunity by 1) residual antibodies from high density infections inhibiting *in vitro* growth or 2) a fast growing parasites infecting immune individuals but the growth only being observable when removed and placed into culture. Independent of density, we found evidence for separate independent associations between febrile illness and complexity of infection with isolate stability *in vitro*. Using qPCR we found that complex infections provided a density stabilizing dynamic in the cultures: single clone infections could growgrew faster but were more likely to die off. Although there was no directly predictability of ex-PMR or culture success using parasite genotype, our results suggest that host immunity

is limiting the growth parasites *in vivo* and that complex infections are more successful in culture despite there being some competition *between genotypes in vitro*.

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THE NANO-TERRAIN OF *PLASMODIUM FALCIPARUM* AND *P. MALARIAE* INFECTED RED BLOOD CELLS ISOLATED FROM CLINICAL SAMPLES

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Atomic Force Microscopy was used to characterize the nano-terrain of PCR-confirmed *ex vivo*-matured isolates of *Plasmodium malariae* and *P. falciparum* from Thailand. The surface of *P. malariae* infected cells are covered with dense 'spike-like' excrescences (mean height: 8 nm; mean diameter: 50 nm), which are morphologically distinct from the larger, more rounded 'knob' structures found on a *P. falciparum* infected red blood cell (mean height: 20 nm; mean diameter: 90 nm). The 'knobs' on red blood cells containing mature asexual forms of *P. falciparum* assist the infected cells to bind/sequester to the vascular endothelium under shear flow conditions and thus, avoid splenic clearance. The function of the *P. malariae* spikes (which were observed on every sexual and asexual stage examined) remains unknown, but it is unlikely to be used for cytoadhesion because there is no evidence that this parasite sequesters.

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FETAL AND MATERNAL HEMODYNAMICS DURING ACUTE MALARIA: PERSISTENT MATERNAL TACHYCARDIA AFTER RECOVERY FROM MALARIA

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Studies on malaria in pregnancy often focus on the effects of chronic placental malaria on the foetus. The maternal and foetal hemodynamics during acute malaria ware never studied properly. The time course of the maternal and fetal heart rate (MHR & FHR) and maternal blood pressure (BP) were studied during acute malaria until 56 days after initiation of treatment with artemether-lumefantrine (AL). We examined 38 pregnant women with acute malaria and 39 healthy pregnant control women. Malaria patients were hospitalized until recovery with a minimum of 3 days. FHR was measured every 4 hours on the first day and every 8 h for another two days and thereafter weekly. Maternal vitals were measured every 8 h for 3 days. Control women were examined once a week on out patient basis.Mean baseline characteristics of malaria patients compared to healthy women were respectively: gestational age (wks) 28.8 and 24.6 (p-value 0.006); maximum FHR (bpm) 165 and 158 (p-value 0.054); minimum FHR (bpm) 137.6 and 128.7 (p-value 0.016); mean BP (mm Hg) 75 and 81 (p-value 0.001); pulse pressure (mm Hg) 40 and 42 (p-value 0.3); MHR 107 and 81 (p-value < 0.001); Geometric mean parasite count (/µl) 13795. Complete time series were collected from 29 malaria patients and 29 controls. Maternal body temperature normalized within 24 hours; BP was normal after 72 h. Surprisingly, whereas MHR in control women showed a physiological increase during the evolution of pregnancy of approximately 7 bpm between day 0 and day 56, the initially increased MHR of malaria patients declined to 94 bpm on day 7 and stabilized at this level. There were no pathological CTG records. The mean FHR normalized after 72 h. In conclusion, acute malaria induces maternal and fetal hemodynamic changes that normalize at a different pace after initiation of treatment with AL. Fetal heart rate and BP normalized between days 3 and 7 after initiation of treatment. Surprisingly, maternal heart rate remained elevated. This is yet unexplained.

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A RANDOMIZED STUDY TO COMPARE A FIXED DOSE COMBINATION OF ARTESUNATE PLUS AMODIAQUINE VERSUS A FIXED DOSE COMBINATION OF ARTEMETHER PLUS LUMEFANTRINE IN TREATMENT OF REPEATED UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA ATTACKS OCCURRING DURING TWO YEARS IN CHILDREN IN UGANDA

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Although in high-endemic areas artemisinin combination therapy (ACT) is used repeatedly by patients, very few studies document the safety of multiple ACT administrations. We designed a study to assess the safety and efficacy of repeated administrations of the fixed-dose combination artesunate + amodiaquine (ASAQ) in comparison with artemether-lumefantrine (AL) in consecutive episodes of uncomplicated *P. falciparum* malaria in children. This randomized, investigator-blinded, comparative study was conducted in a rural community of Eastern Uganda from June 2008 to June 2010. Patients under 5 years of age with uncomplicated *P. falciparum* malaria were randomized to receive either ASAQ once daily, or AL twice daily for three days for each malaria episode occurring over a period of 2 years. Treatment intake was supervised only for first episodes. All attacks were monitored until D42. A total of 413 patients were randomized in the two groups (208 ASAQ, 205 AL). During the study period, a total of 6032 malaria episodes were treated. The median number of episodes were 16 and 15 in ASAQ and AL groups respectively. Treatment-emergent AEs were reported during follow-up in 59.8 % of the patients without significant differences between the 2 groups; only one AE in each treatment group was considered as related to treatment. Adverse event of special interest (AESI) were observed in 28 patients (29 episodes); abnormalities in liver function tests were reported in 23 patients (11 ASAQ, 12 AL), and neutropenia in 6 patients (4 ASAQ, 2 AL). All AESI were reversible. Serious adverse events were reported in 25 patients (31 episodes) without any difference between the two treatment groups. Incidence of adverse events did not increase with the repetition of treatment, in either group. Efficacy analysis is ongoing. An unexpectedly high number of malaria attacks were seen in each treatment group. These results confirmed the satisfactory safety profile of ASAQ in comparison with AL, with no issue related to repeated administration.

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CHALLENGES IN ESTABLISHING A COHORT-EVENT MONITORING DRUG SAFETY STUDY IN IFAKARA AND RUFIJI HDSS, TANZANIA

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The recommended artemisinin combination therapy (ACT) for treatment of uncomplicated malaria in Tanzania is artemether-lumefantrine (AL). Although Artemisinin and its derivatives are generally thought to be safe, there is currently little or no data on its safety among populations in Tanzania. In view of this INESS established a phase IV study to evaluate safety of AL through comprehensive pharmacovigilance in large populations with the aim of documenting rare adverse drug reactions and to characterize known effects in 'real-life'. The methodology employed is cohort event monitoring which is observational, longitudinal and prospective. Patients with diagnosis of malaria for whom AL was prescribed were recruited into the cohort from four health facilities in each HDSS. Information on demographics, use of all medicines, mode of diagnosis of malaria, presenting signs and symptoms, co-diagnoses, events suspected as adverse drug reactions, reasons for stopping the drug and cause of death (if any) were collected using standardized questionnaire. They were followed up on 7 to 10 days after AL was dispensed. This report is on the number recruited so far and the challenges in getting the cohort going. 9028 patients were recruited. 9016 (99.8%) completed follow-up on day 7, of which 668 (7.4%) were done by telephone calls. 12 (0.13%) were lost during follow-up. The main challenges encountered are getting enough trained staff to recruit and follow up patients since CEM is quite labour intensive. 38 health providers and 10 field workers were recruited and offered the relevant training in collaboration with regulatory authorities. This helped to overcome the human resource challenge. Another challenge involved is the difficult to reach areas which are cut off especially during the rainy season. Follow up by telephone was adopted for these areas and this helped to reduce number of lost to follow-up. Setting up a cohort event monitoring program takes time and is demanding in terms of human resource. Training is very important in overcoming this. Involvement of all stakeholders and sponsors is a key to success.

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DRUG-DRUG INTERACTIONS BETWEEN PRIMAQUINE AND CHLOROQUINE: STUDIES USING POOLED HUMAN HEPATOCYTES

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The long established potentiation of primaquine's liver stage activity when co-administered with chloroquine is poorly understood. In the present study, we have undertaken a series of *in vitro* experiments using pooled primary human hepatocytes and recombinant isoenzymes, in order to determine whether the roots of this effect lie in the metabolism of primaquine and if there are any dose dependent inhibitory effects with chloroquine. Increasing chloroquine concentration appears to significantly inhibit primaquine metabolism. Following four hours incubation in hepatocytes an apparent dose dependent decrease in carboxyprimaquine production and production of a metabolite at m/z 261, a mass consistent with the primaquine alcohol, was observed with increasing chloroquine concentrations. This suggests that a significant inhibitory effect on the carboxyprimaquine pathway may play a role in the observed potentiation.

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METABOLITE IDENTIFICATION OF THE 8-AMINOQUINOLINE DRUG PRIMAQUINE USING RECOMBINANT HUMAN METABOLIC ISOENZYMES

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Primaquine's mechanisms of activity and toxicity have long been thought to be mediated by one or more metabolic byproducts, and the exact chemical nature of these species and their metabolic pathways is poorly understood. Previous work in our lab has determined that CYPs 2D6, 3A4, and 2C19 and Monoamine Oxidase-A (MAO-A) are major contributors to primaquine metabolism. In the present study, primaquine was incubated with recombinant versions of these enzymes, as well as CYPs 1A2 and 2C9. The samples were analyzed by LC-MS/MS for the purpose of metabolite identification. In agreement with prior literature observations, MAO-A was found to be primarily responsible for the pathway leading to the formation of carboxyprimaquine, the major observed metabolite *in vivo*. A second metabolite at m/z 261, consistent with the primaquine alcohol, was observed after incubation with MAO-A. Other metabolites, m/z 276, consistent with the hydroxylated species largely implicated in primaquine's efficacy/toxicity profile, are mediated by the CYP enzymes, predominantly 2D6.

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SAFETY OF SEASONAL INTERMITTENT PREVENTIVE TREATMENT AGAINST MALARIA WITH SULFADOXINE PYRIMETHAMINE + AMODIAQUINE WHEN DELIVERED TO CHILDREN UNDER TEN YEARS OF AGE BY DISTRICT HEALTH STAFF IN SENEGAL

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Intermittent Preventive Treatment for malaria in children (IPTc) by monthly administration of sulfadoxine-pyrimethamine plus amodiaquine (SP+AQ) is a new strategy for malaria prevention in areas of seasonal transmission. The aim of this study was to evaluate the safety and effectiveness of IPTc when delivered by district health staff on a large scale in a three rural districts in Senegal. IPTc with SP+AQ administered once per month from September to November was delivered by nine health-posts in 2008, 27 health-posts in 2009 and by 46 health- posts in 2010. Doses administered in each village were documented in a register. A surveillance system was established to record all deaths, and malaria cases were diagnosed at health facilities. Surveillance for adverse events that might be drug-related was maintained in three regional hospitals, three district health centres, 55 health posts and through active follow-up at home. Community health workers visited each child one month after the first and second rounds of treatment to check that there had been no severe reactions to the previous treatment, and to give the next round of treatment. Health staff in all facilities were sent SMS messages before each monthly treatment round to remind them to report any adverse events. All health facilities were visited monthly to check and collect adverse event reports. Admission records for all hospital inpatients admitted following IPTc administration were reviewed for evidence of a possible relationship with

drug intake. After 3 years of intervention over 980,000 documented courses of IPTc had been administered by community health workers. High coverage of three courses of treatment was achieved. No serious adverse events attributable to the intervention were reported. IPTc with SP+AQ is safe and well tolerated when implemented on a large scale. IPTc should be considered for implementation as additional malaria control intervention in areas where seasonal malaria continues to cause severe illness and mortality among children.

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STATEVILLE HUMAN STUDIES OF FORTY 8-AMINOQUINOLINES

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The 8-aminoquinoline (8-AQ) antimalarials are the only class effective against *Plasmodium vivax* relapse and *P. falciparum* gametocytes. This class is highly desirable for malaria prevention, malaria control and especially malaria elimination. However, the risk of the hemolytic toxicity has been recognized with 8-AQs. From 1945-49 at Stateville, IL, researchers evaluated, in a clinical research unit, 40 8-AQs for antimalarial efficacy and tolerability. However, in the ensuing years, much of this information was filed away and forgotten. The purpose of this effort is to compile all unpublished efficacy and safety data reports in humans. We identified, collected and extracted the historical data on efficacy and safety of the 8-AQs from unpublished reports by contacting research experts and by searching libraries where data was kept. Data were entered and analyzed in Excel (2007) and SPSS for Windows, version 16. A total of 403 clinical series (n=2,716) were identified. The majority of subjects received pentaquine (n=1,095) followed by pamaquine (n=810), isopentaquine (n=400) and primaquine (n=85). Thirty six additional 8-AQs were studied in 326 case series, including sitamaquine (WR6026). Sixteen compounds had greater than 50% efficacy reported. The most commonly reported adverse effect of 8-AQs was abdominal pain. Three subjects receiving high dose of pamaquine stopped the drug due to severe abdominal pain. Only 4 subjects had hemolytic crisis - two receiving daily pamaquine 180 mg, and two were on daily pentaquine 120 mg and 60 mg (with concurrent administration of quinine and quinacrine, respectively). Neutropenia, postural hypotension, and drug fever were identified as possible new toxicities in this class. No deaths were identified. In conclusion, this clinical safety data of 8-AQ will permit an ongoing effort to understand more clearly the structure activity and toxicity relationships of 8-AQs in humans before undertaking new synthetic efforts.

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IMPACT OF COMBINING INTERMITTENT PREVENTIVE TREATMENT WITH HOME MANAGEMENT OF MALARIA IN CHILDREN UNDER TEN YEARS, IN A RURAL AREA OF SENEGAL

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Current malaria control strategies recommend (i)Early case detection using rapid diagnostic tests (RDT) and treatment with Artemisinin Combination Therapy (ACT) (ii)Intermittent preventive treatment (iii)impregnated bed nets. However, these individual malaria control interventions provide only partial protection in most epidemiological situations. Therefore, there is a need to investigate the potential benefits of integrating several malaria interventions in reducing malaria prevalence and morbidity. We conducted a cluster randomized trial to assess the impact of combining seasonal intermittent preventive treatment in children (IPTc) with home based management of malaria (HMM) by community health workers (CHWs) in Senegal. Eight CHWs in 8 villages covered by the Bonconto health post, (South Eastern part of Senegal) were trained to diagnose malaria using RDT and provide prompt treatment with CoartemTM to children under 10 years. Four CHWs were randomised to also administer monthly IPTc with single dose of Sulfadoxine-Pyrimethamine (SP) plus three doses of Amodiaquine (AQ) in October and November 2010. A total of 1010 children in the 8 study villages were assigned to a weekly home visit by CHWs during 2 months. During each visit, an RDT was performed by CHWs for febrile children. The incidence of clinical malaria episodes was 7.1/100 child months (95%CI (3.7-13.7)) at risk in communities with IPTc+HMM compared to 35.6/100 child months (95%CI (26.7-47.4)) at risk in communities with only HMM (OR=0.20 95% CI 0.09-0.41, p=0.0001). A survey conducted at the end of the transmission season showed that malaria parasite prevalence was lower in communities with IPTc+HMM(2.05% versus 4.6% p=0.03). Adjusted for age groups, sex, *P. falciparum* carriage, prevalence of malnutrition, IPTc+HMM showed a significant protective effect against anaemia also(aOR=0.59 95% CI 0.42-0.82 p=0.02). Combining IPTc and HMM can provide significant additional benefit in preventing clinical episodes of malaria as well as anaemia among children in Senegal.

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MALARIA DIAGNOSIS AND TREATMENT BEHAVIORS AMONG PUBLIC AND PRIVATE SECTOR HEALTH CARE PROVIDERS IN A PHASE IV TRIAL IN NORTHERN GHANA

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Globally, malaria control programmes are threatened by the development of drug resistance to monotherapies necessitating revision of treatment policies. Since artesunate-amodiaquine (ASAQ) replaced chloroquine as the first line treatment in 2002 in Ghana, little has been documented on the diagnosis and treatment behaviours of providers. The study explored this theme in the context of a Phase IV trial in the Kassena-Nankana District of Ghana that seeks to provide safety and effectiveness data on how antimalarials work when delivered outside trial conditions. In-depth interviews were conducted with 18 health care providers in both the public and private sectors and illness narrative interviews with 32 individuals who suffered malaria two weeks prior to the interview. All interviews were audiotaped, transcribed into English and imported into NVivo 8 for content analysis. The data suggests differences in diagnosis and treatment habits between public and private providers. Although both rely on clinical symptoms for the diagnosis of malaria, only public providers reported occasional use of rapid diagnostic tests (RDT) and microscopy for malaria. Public providers blamed non-use on chronic shortages of RDTs while private providers were unfamiliar with that type of diagnostic. Public providers officially stock and prescribe only ASAQ but some unofficially stock other antimalarials for patients who suffer ASAQ side effects. Private providers favor antimalarials other than ASAQ because of their low cost, easy dosing schedule, little or no side effects, and perceived efficacy. Vast disparities exist between recommended practices and actual practices in both sectors. Public providers more commonly adhere to recommended diagnostic and treatment practices, however improvement is necessary in both sectors. Standard protocols for guiding practice that have been introduced in the public sector should be better implemented in both sectors. The need for a regulatory framework to formalize drug distribution and use in both the public and private sectors is long overdue.

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A RANDOMIZED TRIAL OF A NEW FORMULATION OF ARTESUNATE MEFLOQUINE COMPARED TO ARTHEMETHER LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN ADULTS IN SENEGAL

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WHO recommands the use of ACTs for the management of uncomplicated malaria cases. A new dosage of Artesunate/Mefloquine (Artequin™ Mepha LTD) with 25mg/kg of mefloquine has been developed. This new formulation is in accordance with WHO recommendation to avoid the development of *Plasmodium falciparum* mefloquine resistant strains. However, limited data are avalaible about its effectiveness and tolerability. We conducted a non inferiority trial to assess the effectiveness and tolerability of the new formulation of Artesunate/Mefloquine (AS/MF) compared to Artemether-Lumefantrine (AL) in the treatment of adults with uncomplicated malaria in Senegal An opened randomized trial was carried out in the central part of Senegal from September 2010 to January 2011, including adults and adolescent using WHO 2005 protocol for *in vivo* drug evaluation. Eligible patients were randomised to receiver either AL or AS/MF; a clinical and biological follow-up was done until day 28 for all included patients. 50% of them were followed until day 42. End points included the ACPR at J28, incidence of clinical and biological adverse event. In IIT analysis ACPR at day 28 in after PCR correction was 94.9% for the AS/MF group versus 96.7% for AL group (p=0.42). By PP analysis, ACPR at day 28 after PCR correction was 99.3% for AS/MF and 98.6% for AL (p=0.99). Similar results were found at day 42. The non inferiority of AS/MF was demonstrated both in IIT analysis and PP analysis at day 28 and day 42. Any clinical and biological severe adverse event was observed in the two groups. Our results show the good effectiveness, the good tolerance and the non inferiority of Artesunate-Mefloquine combination (Artequin™) dosed with 25mg/kg of mefloquine compared to Coartem®.

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A SYSTEMATIC REVIEW AND META-ANALYSIS OF NON-RANDOMIZED AND RANDOMIZED CONTROLLED STUDIES OF ARTESUNATE AND AMODIAQUINE FOR THE TREATMENT OF UNCOMPLICATED *FALCIPARUM* MALARIA IN AFRICA

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Artesunate+amodiaquine (AS+AQ) is the second most widely used artemisinin combination therapy (ACTs) for uncomplicated malaria. Published and unpublished non-comparative, comparative randomized and quasi-randomized trials conducted between 1999-2010 were identified via electronic and manual searches through MEDLINE, EMBASE, LILACS and CENTRAL. Standard methodologies were applied for selecting trials and assessing quality. Additional information was obtained from the investigators to allow analysing data by site when multiple sites were involved. Primary endpoints were PCR-adjusted and crude parasitological outcomes by Day 28 on the per-protocol dataset. Random effects models were used to aggregate estimates of randomized controlled trials. Of 83 potential studies identified, 59 comparative and non-comparative trials met inclusion criteria. 55 studies (49 comparative at 81 study sites, 6 non-comparative at 10 sites) enrolling 21,330 patients (8,055 on AS+AQ) in 25 (22 African) countries contributed to the Day 28 efficacy analysis. 53 trials specifically recruited children. Target drug doses were generally AS 12mg/kg + AQ 30mg/kg over three days. Crude Day 28 failure rates for AS+AQ varied widely (0%-80%). After genotyping failures averaged 7% (0-39%). Sensitivity analysis produced failure rates of 5.6-7.8%. Of the 49 studies (81 sites) comparing PCR-adjusted Day 28 failure rates, AS+AQ was significantly more effective than AQ (RR=0.27 [95%CI 0.20; 0.33], AS (RR=0.08 [-0.64; 0.79]), chloroquine (RR=0.05 [0.01;0.08]), SP (RR=0.28 [0.16; 0.40]), AQ+SP (RR=0.46 [0.34; 0.57]), chloroquine+SP (RR=0.17 [0.09; 0.25]), and AS+SP (RR=0.64 [0.41; 0.87]) but not significantly different from artemether+lumefantrine 6 doses (RR=1.46 [0.89; 2.06] and dihydroartemisinin+piperaquine (RR=2.20 [-0.12; 4.14]. Only one comparison each was available with artemether+lumefantrine 4 doses and AS+mefloquine. PCR-adjusted results were highly heterogeneous (except for dihydroartemisinin+piperaquine). This review is an updated inventory of available AS+AQ efficacy data. This study differs from the published Cochrane Reviews in that it considers the effect of site and all comparators and uses also non-comparative studies. AS+AQ met the WHO recommended minimum PCR adjusted efficacy of ≥ 90% in most but not all countries in Africa.

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PHARMACOKINETIC INTERACTIONS BETWEEN THE ANTIRETROVIRAL AGENT EFAVIRENZ AND THE ANTIMALARIAL ARTEMETHER-LUMEFANTRINE IN HEALTHY ADULTS

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Drug-drug interactions are common in patients infected with human immunodeficiency virus (HIV), including in the setting of co-infection with malaria. Artemether and lumefantrine (AL) (co-formulated as Coartem) both are metabolized by cytochrome p450 (CYP) 3A4 with artemether converted to active dihydroartemisinin (DHA). Efavirenz, an antiretroviral drug for HIV-1, is a CYP3A4 inducer. The purpose of this study is to investigate the effect of efavirenz on the pharmacokinetic (PK) disposition of artemether and lumefantrine. This study used a crossover open-label design. Those completing sample analysis were blinded to study details. Twelve healthy adult volunteers received 6 doses of AL (80/480 mg) twice daily. After a two-week washout period, all subjects received a 26-day course of efavirenz (600 mg once a day) to reach steady-state, then resumed AL administration for 3 days. Blood samples were drawn following the sixth dose of AL on day 4 and day 31.Non-compartment PK analysis with WinNonlin 5.2.1 was used to calculate PK parameters. Coadministration of efavirenz with AL led to significant decreases in DHA exposure, as measured by t1/2, Cmax, and the area under the plasma concentration versus time curve (AUC). Specifically, Cmax, AUClast, and AUC0-inf decreased by 39%, 46%, and 39% (p<0.05), respectively. Trends toward decreased exposure of artemether were noted during co-administration compared to AL administration alone (AUClast and AUC0-inf decreased by 51% and 34%, respectively). There was no statistical significance for the change in the DHA:artemether AUC ratio (p=0.824). For the long-acting partner drug, lumefantrine, a trend in decreased AUClast and AUC0-inf was noted (21% and 22%, respectively), although results did not reach statistical significance. Lastly, there was no significant effect of AL on efavirenz exposure. Decrease of artemether and lumefantrine exposure in the context of efavirenz deems to be modest in healthy HIV-negative adults and no dose adjustment is suggested.

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CLINICAL MANIFESTATIONS OF NEW VS. RECRUDESCENT MALARIA INFECTIONS IN AN EFFICACY STUDY

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The results of antimalarial drug efficacy studies undergo correction based on genotyping to exclude new infections that occur during the follow up period. This allows researchers to compare efficacy across differing transmission settings. However, censoring new infections may not be optimal for policymakers in developing countries. In the era of artemisinin-based combination therapy, highly effective but short-acting drugs may produce better "efficacy" than drugs with a prolonged post-treatment prophylactic effect. Further information is required to determine if new vs. recrudescent infections have different clinical implications for patients and if differential prevention of one of these types of infections will have greater public health benefit. We extracted DNA from dried filter papers collected from participants with recurrent parasitemia during drug efficacy studies of sulfadoxine-pyrimethamine in Malawi from 1998 through 2005. We used six neutral microsatellites to genotype the initial and recurrent infections and classified recurrent infections as new, recrudescent or indeterminate. Logistic regression and chi-squared analyses were used to compare the rates of fever and anemia, the clinical outcomes of interest. The recurrent infections were equally distributed between new and recrudescent episodes. The risk of fever and anemia were the same in new and recrudescent infections. The final analyses are being completed and precise risk estimates will be reported. Our study results suggest that both new and recrudescent infections should be considered to be treatment failures when countries select a drug for the national treatment policy. The prophylactic value of longer-acting drugs that reduce the risk of new infections should not be discounted in measurements of antimalarial drug efficacy.

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POPULATION PHARMACOKINETICS OF PIPERAQUINE IN CHILDREN AND ADULT PREGNANT AND NON-PREGNANT PATIENTS WITH UNCOMPLICATED MALARIA

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Malaria is one of the most important infectious diseases in the world. One of the most promising new artemisinin-based combination therapies is the fixed oral piperaquine and dihydroartemisinin combination. Children and pregnant women are especially vulnerable to malaria and the fetus is adversely affected. Reports describing food-interactions and pharmacokinetic properties of this combination in different patient populations are limited. Pharmacokinetic studies were conducted in Thailand (98 children and adults; 24 pregnant women and 24 non-pregnant women; 15 fed adults and 15 fasting adults), in Sudan (12 pregnant and 14 non-pregnant women) and in Burkina Faso (236 children). These studies investigated the pharmacokinetic properties of piperaquine after a standard oral three-day fixed dose regimen of dihydroartemisinin-piperaquine in patients with uncomplicated *falciparum* malaria. Dense venous or capillary plasma samples were collected and drug measurements conducted according to published methods. Concentration-time profiles were characterized using nonlinear mixed-effects modeling or non-compartmental analysis. The pharmacokinetic properties of piperaquine will be described using an individual and pooled analysis approach to investigate the impact of food and the pharmacokinetic differences in these populations.

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DETERMINANTS OF ACCESS TO ACTS AND MALARIA DIAGNOSIS: RESULTS FROM A HOUSEHOLD SURVEY IN THREE REGIONS OF TANZANIA

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While a general consensus over the choice of artemisinin based combination therapy (ACT) as the most effective malaria therapy has developed, a solid evidence-base for choosing the best ACT deployment strategies to gain optimal impact on malaria morbidity and mortality does not exist. Countries are now beginning to adopt policies to enhance ACT deployment that fall more or less into two basic paradigms: (i) making ACTs more readily and speedily accessible to patients, or (ii) targeting ACTs to patients shown to have malaria parasitemia. To design strategies to address and balance these goals, a detailed understanding of current treatment seeking patterns and their determinants is required. We therefore conducted large scale household surveys in three regions in Tanzania with varying transmission levels.5,429 households and 20,973 people were interviewed in Mbeya, Mtwara and Mwanza Regions between June and September 2010. All members of each household who were present and reported fever in the previous two weeks were asked about treatment sought, drugs obtained and the cost of this treatment. Additional data collected covered socio-economic status, net ownership and usage, and knowledge of malaria. Fingerprick blood samples were taken to test for malaria parasitaemia and for anaemia in children under five years. We will present results on the following two key outcomes: percent of people with fever who got an ACT (within 24 or 48 hours), and percent of people with fever who got a finger-prick or heel stick test. We will explore the determinants of these outcomes, considering the influence of age, socio-economic status, location, knowledge and treatment source. Finally, we will identify policy implications for strategies to improve ACT access and targeting, focusing on the current role out in Tanzania out of rapid diagnostic tests to public health facilities, and subsidised ACT under the Affordable Medicines Facility-Malaria.

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THROMBOCYTOPENIA IN MALARIA AND PROGNOSTIC UTILITY OF THROMBOCYTOPENIA IN *FALCIPARUM* MALARIA

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The incidence of thrombocytopenia in malaria is 67 - 90% according to various studies. Some studies suggest the possible role of platelets in the pathology of severe malaria while others have found a correlation of platelet count with prognosis. There are only a few studies done on this aspect in India. The aim of the study was to correlate the presence and severity of thrombocytopenia with type of malaria and to assess the utility of the initial platelet count as an independent prognostic marker for severe *falciparum* malaria. This is a prospective, observational study of patients > 18 years admitted in Medicine Department, in a tertiary

care teaching hospital from August, 2006 to July, 2008. Malaria was diagnosed based on clinical features along with positive Quantitative Buffy Coat method (QBC MP) or Thin Blood Smear examination (Giemsa stain). A total of 131 consecutive patients satisfying the diagnostic criteria during the study period were included. The data was then charted and analyzed using the SPSS 11.0 statistical software package for Windows. The Chi-Square test was used for comparative analysis of data of the different groups. Prevalence of thrombocytopenia was 88.3% and 88.6% in *vivax* and *falciparum* malaria respectively. Patients with severe *falciparum* malaria had a statistically significant lower platelet count (p value = 0.01) compared to non-severe *falciparum* malaria. In patients with severe *falciparum* malaria, those with renal failure (p = 0.019) and hyperparasitemia (p = 0.03) had a statistically significant lower mean platelet count compared to non-severe *falciparum* malaria. Patients with involvement of more than one organ system had a lower mean platelet count compared to those with single organ involvement. The conclusions were 1) *Vivax* malaria can also present with thrombocytopenia and complicated malaria. 2) The admission platelet count can be used to estimate the likelihood of complications and severity of malaria. Our study shows that admission platelet count is significantly lower in those who have hyperparasitemia and acute renal failure. 3) The platelet count is comparable between *vivax* malaria and non-severe *falciparum* malaria but significantly lesser in severe *falciparum* malaria.

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ESTIMATING THE NUMBER OF MALARIA INFECTIONS IN BLOOD SAMPLES USING HIGH-RESOLUTION GENOTYPING DATA

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People who live in malaria-endemic areas often harbour several infections at once. High-resolution genotyping can distinguish between infections by detecting the presence of different alleles at a polymorphic locus. However the number of infections may not be accurately counted since parasites from multiple infections may carry the same allele. We (i) propose a method to estimate the number of infections which would otherwise be detected, taking into account the probability of shared alleles and (ii) carry out simulations to determine the circumstances under which the number of infections is likely to be substantially underestimated due to multiple infections bearing the same allele. We use the allele frequencies to estimate the conditional probabilities of observing different numbers of genotypes given the true numbers of infections present. These probabilities are combined in a Bayesian model with the observed frequencies of genotypes and an assumed distribution for the numbers of infections. We evaluate this model using simulation and show that it can estimate the number of infections with reasonable accuracy. Simulations indicate that the problem is not substantial for most datasets. Large disparities between the number of infections and number of observed genotypes were limited to cases with fewer than 20 alleles, fewer than 20 blood samples, a mean number of infections of more than 6 or a frequency of the most common allele of more than 20%.

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IMPACT OF HEALTH FACILITY-BASED INSECTICIDE TREATED BEDNET DISTRIBUTION IN MALAWI: PROGRESS AND CHALLENGES TOWARDS ACHIEVING UNIVERSAL COVERAGE

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High levels of insecticide treated bednet (ITN) use can reduce malaria burden in countries with intense transmission such as Malawi. Since 2007 Malawi has implemented free health facility-based ITN distribution for pregnant women and children <5 years old (under-5s). We evaluated the progress of this targeted approach toward achieving universal ITN coverage. We conducted a cross-sectional household survey in eight districts in April 2009. We assessed household ITN possession, ITN use by all household members, and *P. falciparum* asexual parasitemia and anemia (hemoglobin <11 grams/deciliter) in under-5s. We surveyed 7,407 households containing 29,806 persons. Overall household ITN possession was moderate (59%) with 67% of eligible households (i.e. households with pregnant women or under-5s) owning an ITN and only 40% of ineligible households owning an ITN. ITN use in households who owned at least one ITN was high, with 76% of all household members, 88% of under-5s and 90% of pregnant women using an ITN the previous night. Of 6,116 ITNs, 92% were used the previous night with a mean of 2.4 persons sleeping under each ITN. In multivariable models adjusting for district, socioeconomic status and indoor residual spraying use, ITN use by under-5s was associated with a significant reduction in asexual parasitemia (adjusted odds ratio (aOR) 0.79, p-value 0.03) and anemia (aOR 0.79; p-value 0.04) prevalence. In addition, we explored potential targeted and non-targeted mass distribution campaign strategies as a means to achieve universal coverage. A campaign that distributes 1 ITN per household might achieve near universal coverage at 1.86 ITNs per household or 2.1 household members per ITN. In conclusion, Malawi has made substantial progress in ITN coverage using health facility-based distribution targeting pregnant women and under-5s, but will need to supplement these activities with non-targeted mass distribution campaigns to achieve universal coverage and maximum public health impact.

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RISK FACTORS FOR *PLASMODIUM FALCIPARUM* MALARIA ACQUISITION ABROAD BY UK RESIDENTS IN 2007

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An increasing proportion of malaria cases in UK residents which were acquired in malaria endemic areas are due to *Plasmodium falciparum*, the most virulent form of malaria, resulting in a number of hospitalisations and a small number of deaths (5-16 annually since 1991). Identifying the main risk groups is necessary to design appropriate public health strategies for reducing the number of cases. However, previous studies have found it difficult to account for exposure within malaria endemic areas. Here we estimate the entomological inoculation rate (infectious bites per person per day) to which travellers are exposed and their duration of stay in malaria endemic areas to calculate the probability of acquiring malaria in each country. We then estimate this risk for acquisition of malaria amongst travellers with different purposes of travel and in different age groups to identify high risk groups. A proportional hazards model was fitted to data

on the trips made by cases and the population as a whole, adjusting for the baseline risk in each country and the duration of stay. Both reason for travel and age-group were found to be significant determinants of the risk of acquiring malaria (p<0.0001). Those visiting friends and family and business travellers were at significantly increased risk of acquiring malaria (adjusted hazard ratio (HR) relative to that of holiday makers 6.0, 95% CI 3.5-11.8, p<0.01 and HR 2.3, 95% CI 1.5-7.6, p<0.01, respectively). All age-groups were at lower risk than children aged 0-15 years old (p<0.01). Travellers visiting friends and family, business travellers and young children remain at increased risk of malaria acquisition after adjusting for the risk of infection in their destination and their duration of stay. These groups should be the target of programmes to increase awareness of malaria acquisition when travelling.

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MOLECULAR EPIDEMIOLOGY OF *PLASMODIUM* INFECTIONS IN TWO MALARIA ELIMINATION SETTINGS IN VANUATU AND SOLOMON ISLANDS

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Malaria prevalence in Tanna Island, Vanuatu and Temotu Province, Solomon Islands has declined significantly due to effective malaria control programs and both have a commitment to provincial malaria elimination. As the first step toward elimination, mass blood surveys were conducted to obtain baseline epidemiology information. From these surveys, microsatellite genotyping was used to assess the number of parasite haplotypes being transmitted, their frequency, diversity and distribution. For *Plasmodium vivax*, genotyping revealed 22 haplotypes of the 75 isolates typed for Tanna, and 46 haplotypes of the 82 isolates typed for Temotu. The number of *P. vivax* haplotypes was approximately 4 times greater than that of the sympatric *P. falciparum* populations. In Tanna, while most *P. vivax* haplotypes were scattered on the island, some appear to be clustered to small regions indicating some limitation in human movement. In Temotu, no clustering was observed. Parasite population structure for both locations were analysed. The results provide good epidemiological data for both locations, and may reflect the malaria transmission levels. These data can be used to monitor any change in malaria epidemiology and transmission, as well as progression toward elimination.

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MOLECULAR EPIDEMIOLOGY OF MALARIA IN SOUTHEASTERN BANGLADESH, WITH THE MAIN FOCUS ON THE SYMPATRIC DISTRIBUTION AND DIAGNOSIS OF *PLASMODIUM OVALE WALLIKERI* AND *P. OVALE CURTISI*

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In spite of the high prevalence of malaria in Southeastern Bangladesh, there remains a significant shortage of information regarding the presence of four out of six human malaria parasites: *Plasmodium ovale wallikeri, P. ovale curtisi, P. malariae* and *P. knowlesi.* The molecular epidemiology of these pathogens as well as of the more prevalent species *P. falciparum* and *P. vivax*, were investigated within the course of field surveys and a hospital-based survey in Bandarban District in Southeastern Bangladesh between 2006 and 2010. Filter paper samples from 1,867 asymptomatic participants and 379 patients presenting with symptomatic febrile illnesses were analyzed using a genus- and species specific nested PCR method, targeting the small subunit ribosomal RNA (SSU rRNA) gene. Samples positive for *P. ovale* spp. were further analyzed by multilocus sequence analysis of 3 loci (cox1, SSU rRNA, porbp2) and the comparison of several different PCR techniques targeting the SSU rRNA and PoTRA genes for their accuracy regarding the diagnosis of *P. ovale* spp. In the course of this study a new PCR method was established and tested for its accurate diagnosis of *P. ovale*. In both, symptomatic and asymptomatic participants, *P. falciparum* was the dominant species, followed by *P. vivax* and the less prevalent parasites *P. malariae* and *P. ovale* spp. We found a high rate of mixed infections and asymptomatic malaria cases in this region. However, there was no indication of the presence of *P. knowlesi* in Southeastern Bangladesh. Our data provide the first evidence of *P. ovale wallikeri* and *P. ovale curtisi* in Bangladesh and their sympatric distribution in South Asia.

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THE DISTRIBUTION OF HUMAN *PLASMODIUM* SPECIES IN CENTRAL VIETNAM IS COMPLEX WITH MARKED AGE-DEPENDENT PREVALENCE OF SYMPTOMATIC AND PATENT INFECTIONS

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In Vietnam, *Plasmodium falciparum* and *P. vivax* are responsible for the majority of malaria infections while *Plasmodium malariae* and *P. ovale* infections are rarely reported. Nevertheless, a species specific PCR analysis on 2,303 blood samples, collected during a cross sectional survey carried out in a forest area of malaria endemic province in central Vietnam, identified 223 (prevalence = 9.7%) *P. falciparum*, 170 (7.4%) *P. vivax*, 95 (4.1%) *P. malariae*, and 19 (0.8%), *P. ovale* mono-infections with mixed infections occurred at and 164 (7.1%) mixed infections. Out of the 671 positive samples positive by PCR (prevalence sub-patent infections=29%), only 331 (50%) were positive also by microscopy. *P. malariae*, *P. ovale* and mixed infections were poorly diagnosed by microscopy. Although, clinical and sub-clinical infections occurred in all age groups, the risk of infection and disease decreased significantly with increasing age. The parasite densities and the prevalence of patent infections were significantly lower in the adult population, probably due to the acquired partial immunity. The common occurrence of sub-patent infections seems to indicate that the malaria burden is largely underestimated which calls for the urgent development of improved diagnostic and surveillance tools for future elimination perspectives and that diagnostic and therapeutic policies should be adapted accordingly.

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ESTIMATES OF THE IMMUNE PARAMETERS DETERMINING THE DURATION OF ANTIBODY RESPONSE TO *PLASMODIUM FALCIPARUM* INFECTION IN GHANAIAN INFANTS

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Infants in regions of intense malaria transmission are particularly vulnerable to malaria in their first years of life before they have acquired substantial immunity. Understanding the processes underlying the acquisition and loss of immune effector mechanisms is crucial for understanding the epidemiology of the disease and the likely impact of vaccines. From a longitudinal study of 151 Ghanaian infants followed for over 2 years we fitted biological models of immunological processes to IgG antibody titres to five *Plasmodium falciparum* antigens using nonlinear mixed effects models to investigate the acquisition and loss of humoral

immune responses. We assume that exposure to *P. falciparum* antigen induces proliferation of antigen-specific B cells and their differentiation into IgG antibody secreting plasma cells and that the duration of the IgG response is dependent upon (i) the half life of individual IgG molecules, (ii) the half life of IgG secreting cells and (iii) rates of reinfection which induce differentiation of new populations of memory B cells and IgG secreting cells. For apical membrane antigen 1 (AMA1) we estimate that maternally-acquired antibodies have a half-life of 72 days (95% CrI 64-82); exposure induced antibodies have a half-life of 152 days (95% CrI 126-186); and ~2% of antigen-specific B cells differentiate into long-lived memory B cells. For merozoite surface protein 1 (MSP1) we estimate that maternally-acquired antibodies have a half-life of 64 days (95% CrI 55-75); exposure induced antibodies a half-life of 57 days (95% CrI 49-79); and ~1% of antigen-specific B cells differentiate into long-lived memory B cells. These results demonstrate that whilst circulating antibodies decay over a period of weeks as measured in other studies, antigen-specific memory B-cells may circulate for months to years providing a degree of immunity to re-infection.

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THE IMPORTANCE OF CELL PHONE IMPLICATION AS A DYNAMIC TOOL TO GET MALARIA POSITIVE CASES AT REMOTE KUHALONG AND RAJBILA UNIONS IN BANDARBAN DISTRICT, BANGLADESH

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The tribal rural Chittagong Hill Tracts of Bangladesh is where malaria has highest prevalence rate and 1.5 million people are at risk. The ICDDR, B in collaboration with Johns Hopkins Bloomberg School of Public Health is conducting a malaria epidemiological study in two adjacent unions comprising a population of 20,000 individuals in an area over 172 square kilometres. About 20% of the communities are densely forested and have no road access. The recent introduction of cell phones two years ago in the rural areas of Bandarban district has opened new doors to using this technology for epidemiological purposes as more than one third (1401) households in this region own at least one cell phone or are able to borrow one. The study subjects have been instructed to phone the study physician with fever and associated symptoms of malaria. Villagers know that they will receive free medicine and latest RDTs to identify malaria. From June 2010 until May 2011, there were 211 phone calls for potential malaria cases. Of the calls, a total of 54 were malaria-positive cases, which represents 27% of all 201 malaria-positive cases in the study unions since June 2010. In addition, a severe malaria patient in a remote area was saved because of the cell phone initiated the 2.5 hrs drive to a health facility. Costing only a few taka (cents) per call, represents a significant portion of the total number of positive cases, highlighting the importance of cell phones in this type of epidemiological activity. This method of immediate medical attention to malaria-positive patients is absolutely critical when considering strategies for malaria eradication in Bangladesh, and should be applied to other malaria endemic districts if the resources are available. Using current cell phone technology in the medical and scientific realms is effective, efficient, and should entice phone companies to broaden their coverage to all rural areas of the country.

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MAPPING MALARIA RISK IN CÔTE D'IVOIRE

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In Côte d'Ivoire, an estimated 767,000 disability-adjusted life years are lost annually due to malaria the disease remains of great public health importance, ranking the country at position no. 14 with regard to the global burden of malaria. The purpose of this study was to predict malaria infection risk in Côte d'Ivoire for children aged <16 years. A geostatistical modeling approach was employed using point-prevalence data from published and unpublished work. Four Bayesian regression models, two of which were spatially-explicit, were fitted for *Plasmodium* spp. infection prevalence using a suite of environmental covariates (i.e. elevation, distance to water bodies, maximum land surface temperature and rainfall). Model fits were compared with the deviance information criterion. A total of 235 georeferenced *Plasmodium* prevalence data points from surveys including children aged <16 years were obtained from published and unpublished work carried out between 1988 and 2007. The majority of data points (n=182, 64 %) were collected between 2000 and 2007, whereas the remaining 53 data points were obtained from surveys between 1988 and 1999. The best fitting model was a Bayesian non-stationary regression model, with rainfall and land surface temperature identified as significant covariates. This model was used for prediction and mapping of *Plasmodium* spp. infection risk at non-sampled locations. The obtained malaria risk map can be utilized for spatial targeting of control interventions, which is important for the national malaria control program in Côte d'Ivoire.

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MALARIA ATTRIBUTABLE FRACTION OF FEVER ACCORDING TO SEASON IN A MALARIA VACCINE TRIAL SITE OF BURKINA FASO

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In Burkina Faso, malaria management policy recommends presumptive treatment of malaria in children under five years presenting at the peripheral health facility with fever. This policy facilitates early management of malaria, but could also delay the care of other etiologies of fever such as septicemia and increased risk of child mortality. This study aims to estimate the malaria attributable fraction of fever in a vaccine trial site of Burkina Faso. We conducted two community-based cross-sectional surveys in children aged 0 months to 5 years of age from four villages of the health district of Saponé. The first survey was conducted during the rainy season and the second in the dry season. Parasitological and clinical examinations were performed. A fever case was defined as objective temperature ≥37.5 °C or history of fever in the past 24 hours. Fever was more prevalent during the rainy season (91/487; 18.7%) than the dry season (73/522; 14.0%). The malaria attributable fraction of fever presented the same trend with 41.9% during the rainy season and 34.5% during the dry season. The alternative parasite thresholds for the malaria case definition that achieved optimal sensitivity and specificity (70-80%) were 1350 parasites/µl during the low season and 3150 parasites/µl during the high season. Our results confirm that malaria is a main cause of fever in the Saponé health district. The relationship between fever and parasitaemia depends on the season. Burden of the disease is

higher during the malaria high transmission season. Malaria management policy should recommend the use of microscopic or at least RDT, before administration of anti-malarial drugs during the dry season.

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MARKERS OF INFECTION AND EXPOSURE TO MALARIA IN AFGHAN REFUGEE CAMPS IN KHYBER PUKHTOON-KHWA (KPK) PAKISTAN

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In South Asia, Pakistan is amongst the most malarious countries and large-scale epidemics have been reported in the past. Khyber Pukhtoon-Khwa (KPK), Northern Pakistan is characterised by seasonal transmission with predominantly *Plasmodium vivax* malaria. The high number of Afghan Refugees in KPK has compounded the malaria problem as the malaria incidence in this group is relatively high. Therefore this province needs an effective and sustainable programme to achieve the overall objectives of malaria control. The project investigated the use of sero-prevalence to measure transmission intensity and to identify the risk factors in KPK. The study may provide additional insight into historical patterns of malaria transmission, the dynamics and kinetics of immunity in the population. KPK is low endemic area and suitable for malaria elimination, but assessing transmission is difficult because of lack of sensitivity of commonly used methods. We used serologic markers to detect variation in malaria exposure in these refugee camps. This information will help to guide researchers and decision-makers in targeting intervention efforts. A cross-sectional survey was conducted in five Afghan refugee camps of Khyber Pukhtoon-Khwa (KPK), Northern Pakistan between June and September in 2010. Blood samples were obtained on filter paper from three household members to measure parasite prevalence, transmission intensity and exposure using serology (ELISA). Infection status was determined by Rapid Diagnostic Test (RDT).Sub-samples are also assayed using molecular techniques (PCR) to identify low density parasite infections. PCR results will allow serological data for examination of the effect of parasite carriage on seropositivity and evaluation of RDT. Data will be presented on age sero-prevelance of *Plasmodium falciparum* and *Plasmodium vivax* merozoites surface proteins ($MSP1_{19}$). Parasite rate will be assessed by RDT and PCR for both *Plasmodium falciparum* and *vivax*. Results will provide an up to date insight in to the region's malaria transmission intensity.

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EFFECT OF MALARIA TRANSMISSION SEASON ON HEMATOLOGIC MEASUREMENTS IN HEALTHY MALIAN CHILDREN LIVING IN A MALARIA ENDEMIC AREA

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Hematological indices are commonly used in epidemiological studies of malaria: e.g., use hemoglobin (Hb) values to define anemia, calculate parasitemia based on the number of white blood cell (WBC) on a slide, etc. However, few studies report the effects of malaria transmission season on the hematological measurements. Therefore, we collected venous blood from 249 Malian children aged 3-12 years living in a village where malaria transmission is seasonal. Red blood cell (RBC) count, Hb, hematocrit (Ht), WBC count, and WBC subsets were determined in May 2010 (beginning of the transmission season) and January 2011 (end of the season). At the time of blood collections, all of the children appeared to be healthy, and none of children in May and 19% children in January were determined as *Plasmodium falciparum* positive microscopically. At the beginning of the season, older children showed higher median Hb levels (10.9 g/dl for 3-5 years vs. 11.8 for 9-12 y, $p<0.001$) and Ht (36% for 3-5 y vs. 38.1 for 9-12 y, $p<0.001$), while RBC counts were the same regardless of age. On the other hand, older children showed lower WBC counts (7.81 x 10^3/ml for 3-5y vs. 5.72 x 10^3 for 9-12 y, $p<0.001$). The children experienced an average of 1.1 malaria episodes during the transmission season. From May 2010 to January 2011, the children showed higher levels of RBC (4.51 to 4.68 x 10^6/ml, $p<0.001$), Hb (11.3 to 11.8 g/dl, $p<0.001$) and Ht (36.9 to 39.0 %, $p<0.001$), while the level of WBC was lower in January (6.62 to 5.96 x 10^3/ml, $p<0.001$). There were no correlations between the changes in the hematological indices and the number of malaria episodes experienced during the transmission season. Relative numbers of WBC subsets also changed from May 2010 to January 2011; 34 to 42 % neutrophils, 6.6 to 7.7 % monocytes and 54 to 45 % lymphocytes ($p<0.001$ for all of comparisons). Although our study did not uncover the mechanisms responsible for the changes, the results suggest that it is inappropriate to assume that hematological values are constant regardless of age and/or season.

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PARTURIENT KENYAN WOMEN ACCURATELY SELF-REPORT MALARIA AND BENEFIT FROM SELF-REPORTED USE OF SULFADOXINE-PYRIMETHAMINE BUT ARE ALSO AT RISK FOR HIGH DENSITY PLACENTAL INFECTION

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Using a large cross-sectional study (n=1,082) conducted with mothers at parturition in western Kenya, associations between self-reporting of malaria (SRMal) and clinical diagnosis of placental malaria (PM), and between medication use, specifically sulphadoxine-pyrimethamine (SP), and maternal and fetal outcomes were investigated. A strong correlation was found between SRMal and microscopic diagnosis of PM (OR: 2.69 CI: 1.57-4.60). Although SP users had a reduced risk overall for PM (OR: 0.60 CI: 0.35-1.02) and had longer gestations (OR: 1.52 CI: 1.11-2.09), those SP users who had PM were more likely to have high levels of parasitemia (OR: 3.87 CI: 1.33-11.27). The results show SRMal in areas holoendemic for malaria can be used to inform decisions about treatment of pregnant women when resources are limited and laboratory diagnosis is unavailable. Furthermore, while SP appears efficacious in protecting against PM and poor birth outcomes, drug failure may contribute to risk for high density placental parasitemia.

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SINGLE DOSE MASS DRUG ADMINISTRATION OF AZITHROMYCIN DECREASES MALARIA INCIDENCE IN A LARGE COHORT TREATED FOR OCULAR TRACHOMA

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Single dose mass drug administration of azithromycin (AZ MDA) reduces community wide trachoma rates and all-cause mortality. We investigated the malaria protection, and selection for *Plasmodium falciparum* resistance following a trachoma intervention that included single dose AZ MDA.

A cohort of 1,086 participants from AZ MDA treated villages and 1,063 controls was drawn from 8 rural villages in central Tanzania and followed from January through July 2009. Participants from 4 treated villages received single dose azithromycin (20 mg/kg in children, 1 g in adults). Blood samples were taken on filter paper from the participants at baseline, and months 1, 3, 4, and 6. Diagnosis for *P. falciparum* infection was made by detection of 18S ribosomal DNA in real-time quantitative PCR. Data were analyzed with a multivariate logistic regression controlling for self-reported bed net ownership, anti-malarial use, rainfall, and altitude. Random effects were used to account for clustering within villages. In the first month after AZ MDA, 13 of 796 (1.63%) participants from AZ MDA villages tested positive for *P. falciparum* compared to 34 of 739 (4.40%) participants in control villages. The odds of incident *P. falciparum* infection decreased 67% (95% CI: 39%, 82%) in AZ MDA treated villages compared to controls in the first month after treatment; however in the ensuing months AZ MDA control and treated villages had similar proportions of infection. Sequencing of the *P. falciparum* ribosomal L4 protein from more than 50 clones from a dozen patients indicates no changes consistent with drug resistance to azithromycin. These data provide evidence that AZ MDA retains a transient anti-malarial prophylactic effect, and that AZ MDA has not selected for resistance mutations to the ribosomal L4 protein target in a region with a ten-year history of azithromycin use for trachoma.

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BASIC EFFICACY OF ORAL INSECTICIDES IN TOXIC SUGAR BAITS TO CONTROL MOSQUITOES AND SAND FLIES IN THE LABORATORY

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The use of oral insecticides in sugar solution is a promising method that requires good basic oral efficacy against target species. A series of feeding experiments in the laboratory was performed to test the palatability of toxic sugar baits (TSB) and the basic efficacy of oral insecticides (spinosad, thiamethoxam, dinotefuran, boric acid) in TSB against male and female sand flies (field-collected *Phlebotomus sergenti* and laboratory-reared *P. papatasi*) and mosquitoes (*Culex pipiens, Anopheles stephensi* and *Aedes aegypti*) exposed to a series of insecticide dilutions for 24 h. Cumulative mortality rates were determined at 24, 48 and 72h post-exposure, and LC values were calculated. The persistence of TSB was tested in field conditions in Israel. Flies fed on all types of baits tested, and neither insecticide deterred feeding. Mortality was dose-dependent and faster on TSB with thiamethoxam or dinotefuran than TSB with spinosad or boric acid. Feeding and mortality rates differed between old and young age cohorts. Residual persistence of TSB highlighted important differences between the insecticides. Their suitability for potential use in TSB for vector control will be discussed.

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HOW SHOULD NOVEL VECTOR CONTROL TOOLS BE TESTED?

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Long lasting insecticidal nets (LLINs) were developed and recommended by the World Health Organization in the early 2000s, which enabled the massive scale-up of vector control that has been seen in recent years. Today, insecticidal tools form the basis of vector control interventions in many disease control initiatives, including the Global Malaria Action Plan that strives for Universal Coverage of at-risk populations using locally appropriate tools such as LLINs or indoor spraying of residual insecticides. To date, pyrethroids are the only class of insecticide approved by the WHO for use on mosquito nets, for reasons of safety, efficacy, acceptability and cost. The development of insecticide resistance in mosquito vectors is of increasing concern and evidence on the extent of the resistance problem is mounting. Reports of reduced efficacy of pyrethroid-treated nets in several countries with documented insecticide resistance underscore the need for new paradigms in vector control. Much of the research on the efficacy of non-pyrethroid insecticides on nets has been performed in experimental huts, which can be used to compare one product with another at a certain point in time and space. However there is very limited published information relating results from experimental hut trials to the effectiveness of vector control tools at village level. New vector control interventions will differ greatly in their mode of action, efficacy, features and applicability in different settings but new and specific categories for novel disease control tools are required. Clear testing guidelines for new categories of interventions will help to drive innovation by clearly defining the product requirements and a providing a clear pathway to a market that will recognise the value of those novel products.

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ESTABLISHMENT OF AN INSECTICIDE RESISTANT COLONY OF *ANOPHELES GAMBIAE* FOR THE ASSESSMENT OF NEW VECTOR CONTROL TOOLS

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Vector control today is heavily dependent on insecticide-based interventions, many of which are based on pyrethroids for example insecticidal nets, indoor residual house spraying and new products such as durable wall lining. Current product evaluation guidelines rely on standard susceptible laboratory strains, such as the KISUMU strain of *Anopheles gambiae* for efficacy testing of interventions. However many countries are now reporting insecticide resistance resulting in an increasing need to test new vector control tools in the laboratory using a 'standard' resistant mosquito strain. A field collected strain of *An. gambiae*, resistant to the main insecticides used in public health and agriculture (e.g. pyrethroids, organochlorines, carbamates and organophosphates) from Tiassalé village in Ivory Coast was colonized and maintained in the laboratory over 12 generations. The strain was fully characterized using the WHO susceptibility test, Hot Oligonucleotide Ligation Assay (HOLA) for East- and West-African *kdr* mutation assessment and an indirect determination of metabolic resistance using bioassays with a range of synergists. Mortality rates from WHO susceptibility tests revealed a strong level of resistance to pyrethroids (deltamethrin = 65.33%, permethrin = 45.58%), organochlorines (DDT = 4.10%), organophosphates (fenitrothion = 86.48%) and carbamates (bendiocarb = 33.82%, propoxur = 13.88%). After 12 generations in the lab without selection, the resistance levels to these compounds were higher than at the beginning (deltamethrin = 17.50%, permethrin = 2.50%), organochlorines (DDT = 2.63%), organophosphates (fenitrothion = 54.43%) and carbamates (bendiocarb = 0%, propoxur = 2.59%).The various insecticide resistance mechanisms investigated included target site resistance (*kdr* mutation) and metabolic resistance and will be discussed in the context of how this strain can be used for estimating the efficacy of new tools.

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THE "AUTO-DISSEMINATION" APPROACH: A NOVEL CONCEPT TO FIGHT *AEDES ALBOPICTUS* IN URBAN AREAS IN ITALY

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In the last 20 years, *Aedes albopictus* has become a permanent pest and an intractable nuisance problem in urban and peri-urban areas of most Italian regions. In the last year, the species has also colonized other parts of Europe and now presents a significant public health risk, as shown by the 2007 Chikungunya outbreak in Emilia Romagna (Italy). Most municipalities in Northern and Central Italy, where *Ae. albopictus* reaches its highest densities, carry out expensive control campaigns which aim to decrease larval (and thereby adult) densities by treating urban drainage systems with larvicides. The success of these interventions is hampered by the sheer number of alternative breeding sites available and by *Ae. albopictus*' propensity to subdivide their progenies among different unstable aquatic habitats in order to maximise their chances of successful development (skip oviposition). The difficulty in targeting these habitats is also increased by the significant presence of small and medium sized water containers in private gardens, courtyards and terraces. We will present the results of laboratory and field experiments carried out in Rome to test whether the "auto-dissemination" approach successfully exploited against *Ae. aegypti* in Peru (reported previously) could be applied to interfere with the development of *Ae. albopictus* larvae in a n Italian setting as well. This method exploits wild adult mosquitoes to disseminate a juvenile hormone analogue between contaminated resting sites and oviposition sites. The distribution of the hormone in the mosquito breeding sites is therefore very accurately and efficiently targeted. Moreover, the technique is interesting because of the amplification in coverage seen between resting and oviposition sites. Results showed significant larval mortality in sentinel versus control larval sites, confirming that the "auto-dissemination" approach has a very good potential as a novel control strategy, and that it may allow unparalleled coverage of aquatic *Ae. albopictus* habitats in urban areas of Italy, as well as of other countries.

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CO-DEPLOYMENT OF WHO ASSAY AND CDC BOTTLE ASSAY FOR INSECTICIDE RESISTANCE SURVEILLANCE IN ZAMBIA

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Insecticide resistance is determined primarily by one of two roughly equivalent *in vivo* assays the "WHO tube assay" and the "CDC bottle assay". Both are limited by availability of mosquito specimens and the skills needed to conduct the tests and interpret the results. The WHO assay is widely employed but its cost, storage and shipment requirements pose limitations for use in remote endemic areas. A study to evaluate the possible emergence of insecticide resistance in malaria vectors *Anopheles gambiae* s.s to two classes of insecticides, Organo Chlorines and pyrethroids, was conducted in Mushili-Commando and Chipulukusu compounds of Ndola District between 2009 and 2010. The study was conducted using the established CDC bottle assay protocol and presented evidence of resistance to DDT, permethrin, deltamethrin, and suspected resistance to lambdacyhalothrin and alpha-cypermethrin. The findings where validated by using the WHO tube assays. Both assays showed high levels of DDT and permethrin resistance. Mortality at diagnostic time was between 3% and 17%, and 8% and 51% for DDT and permethrin respectively. The standard WHO discriminating dosages showed 23.7% and 43% mortality for DDT and permethrin respectively. The emerging problem of insecticide resistance in Zambia threatens the future effectiveness of indoor residual spraying (IRS) and Long Lasting Insecticidal nets (LLITNs), and necessitates intensive resistance surveillance. For sustainable establishment of robust resistance monitoring in operational research to strengthen malaria control and elimination efforts, simple and affordable methods, with parsimonious reagent and equipment requirements are essential. To maximize the operational monitoring of insecticide resistance in vector populations, Zambia has adopted the use of both assays in spatially segregated areas, the CDC bottle assay for routine monitoring in rural remote areas while WHO assays are utilized in areas with ease of access to entomological laboratories and are employed for validation of CDC bottle assay results precedent to policy decision making.

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INSECTICIDE SUSCEPTIBILITY STATUS OF *ANOPHELES GAMBIAE S.L.* IN NORTHERN UGANDA

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Larger scale chemical vector control programs in Africa working on malaria prevention and control have had a growing concern regarding the development and spread of malaria vector resistance to public health insecticides. A study conducted in six districts of Northern Uganda in 2010, collected vector-insecticide resistance data to inform decision making about which insecticide would be unaffected by existing vector-insecticide resistance patterns in the area. *Anopheles gambiae s.l.* were collected from each district and tested using a WHO insecticide susceptibility test. The insecticides tested included Carbamate (Bendiocarb), which killed 100% (n= 600) of the vectors tested, a result suggesting 100% susceptibility in all districts. Pyrethroid (Alpha-cypermethrin) caused 100% mortality (n= 400) in vector collections from four districts (Amuro, Apac, Oyam and Gulu) and a reduced susceptibility of 74.5% (n=420) in the remaining two districts (Kitgum and Pader). This study also confirmed reduced susceptibility with 30% mortality to DDT (n=460) in all districts. During following year, Organo Phosphate (Pirimiphos-methyl) and Bendiocarb were tested instead of Alpha-cypermethrin and DDT. Both insecticides produced 100% mortality (n=100 each) in all districts. These results indicate high levels of DDT resistance and reduced susceptibility to Alpha-cypermethrin in the target vector population and argue for the use of both classes of insecticides for malaria control in the region. The alternative, rotational or mosaic use of Bendiocarb and Pirimiphos-methyl in vector control programs could mitigate the likelihood of insecticide resistance in future. This study demonstrates the utility of information from routinely monitored vector-insecticide susceptibility tests to identify candidate insecticides for future vector control programs and to design resistance management strategies for IRS, LLITNs and other chemical vector control programs in the region.

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PLASTICITY AND HERITABILITY OF *IN VITRO* SPATIAL REPELLENCY BEHAVIORAL RESPONSES IN *AEDES AEGYPTI* MOSQUITOES

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There is currently much interest in the potential to develop novel vector control interventions based on spatial repellency (SR) to combat disease transmission at sites that are unaffected by traditional tools such as indoor residual spraying (IRS) and bed nets (ITNs). Despite the fact that behavioral modification as a means to disease reduction has been recognized for

more than 60 years, the underlying mechanisms of SR behavior remain poorly understood. Of particular relevance to a field-based intervention intended to exploit SR is the need to describe the behavioral plasticity and heritability of SR in the target vector(s) through generations - will there be selection for responders over time? We present results on 1) the reproducibility of behavioral responses observed in individual cohorts of mosquitoes exhibiting both positive (responders) and negative (non-responders) SR behavior and 2) selective breeding experiments designed to illustrate the heritability of SR based on the proportion of SR responders in subsequent mosquito cohort generations. Studies were performed under laboratory conditions against *Aedes aegypti* (THAI strain) mosquitoes exposed to varying spatial repellent products.

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REDUCED SUSCEPTIBILITY TO PYRETHROIDS IN *ANOPHELES GAMBIAE* POPULATIONS OF WESTERN KENYA

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As malaria control interventions directed against *Anopheles* vectors increase in sub-Saharan Africa, it is crucial to assess the sensitivity of the mosquito vectors to the insecticides used in the programs. The use of ITNs is widespread in western Kenya with up-scaling of IRS in targeted districts. Therefore, we investigated the susceptibility of *An. gambiae* and *An. arabiensis* to pyrethroid and carbamate insecticides. Adults for analysis were reared from field-collected larvae or from eggs of wild-caught females, from Ahero, Budalangi, and Bungoma. Phenotypic resistance was determined using WHO test kits and time-dependent, bottle bioassays. Microplate enzyme assays were done with insecticide-exposed and non-exposed mosquitoes to investigate whether detoxifying enzymes were over-expressed compared to controls. The frequency of knockdown resistance (KDR) alleles was determined by RT-PCR while species were identified using standard PCR in 3,396 mosquitoes. The Bungoma sample was comprised of 74% *An. gambiae* s.s. and 26% *An. arabiensis* (n=904). About 99.4% of the *An. gambiae* s.s. were homozygous for the KDR genotype while the allele was absent in *An. arabiensis* (n=251). *An. gambiae* s.s. populations from Bungoma showed phenotypic resistance to permethrin (62%), deltamethrin (34%) and moderate resistance to bendiocarb (12%). Samples from Budalangi were also moderately resistant to permethrin (26%) and had slightly reduced sensitivity to deltamethrin (15%). There was no significant difference between the CDC Bottle and WHO tube bioassay methods suggesting that either method can be used to accurately quantify insecticide resistance. *An. arabiensis* were susceptible to all the three insecticides and none had any of the KDR genotypes. Permethrin resistant *An. gambiae* s.s. from Bungoma 1.8 fold elevation in nonspecific esterases most likely due to the high frequency of organophosphate insecticides used in the area and thus may be responsible for insecticide resistance in synergy with kdr.

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MOLECULAR MECHANISMS OF PYRETHROID RESISTANCE IN FIELD POPULATIONS OF *ANOPHELES FUNESTUS*, MAJOR MALARIA VECTOR IN AFRICA

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Although more cases of insecticide resistance are being reported in field populations of *Anopheles funestus* in Africa, the underlying molecular mechanisms remained uncharacterised contrary to the other major malaria vector *An. gambiae*. To fill this gap in our knowledge, we have been investigating mechanisms of pyrethroid resistance in field populations of this species from different regions of Africa. Different resistance patterns have been observed between *An. funestus* populations from different regions in Africa. Pyrethroids/carbamate resistance is observed in Southern Africa, Pyrethroids/DDT in East, Pyrethroid/Carbamate/DDT in West and DDT/Dieldrin in Central Africa. To generate new genomic tools to investigate mechanisms of resistance in this species, the transcriptome of *An. funestus* was sequenced using 454 pyrosequencing and generated 18,000 ESTs used to design a whole genome microarray chip. By comparing pyrethroid resistant field populations to susceptible samples in microarray analysis, we identified that resistance is mainly conferred by two duplicated P450 genes CYP6P9a and CYP6P9b with varying involvement depending of the country. Other detoxification genes such as CYP6Z1, CYP6P4a/b plus others genes seems also to be playing a role in the resistance. This microarray result was confirmed by qPCR. RNA interference confirmed the involvement of CYP6P9a/b in the resistance. CYP6P9a/b were also shown to metabolise pyrethroid *In vitro* using a recombinant enzyme of each copy. Polymorphism analysis between resistant and susceptible identifies significant differences and analysis is currently carried out to determine the causative ones. Analysis of haplotypes of the voltage gated sodium gene associated with the knockdown target site resistance indicated a correlation with pyrethroid resistance but the common L1014F mutation was not identified after screening samples from 7 countries. The characterization of these resistance mechanisms in *An. funestus* will help to improve the implementation and management of future malaria vector control programs in Africa.

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AN IMPROVED AUTOCIDAL GRAVID OVITRAP (CDC-AGO) FOR THE CONTROL AND SURVEILLANCE OF *AEDES AEGYPTI*

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Limited success has been achieved using traditional vector control methods to prevent the transmission of dengue viruses. Integrated control programs incorporating alternative tools, such as gravid ovitraps (lethal ovitraps, sticky ovitraps) may provide a greater potential for reducing vector populations and dengue transmission. We developed the CDC autocidal gravid ovitrap (CDC-AGO) as a simple, low-cost device for surveillance and control of *Aedes aegypti* without the use of pesticides that does not require servicing for an extended period of time. In a previous area-wide, intervention study in southern Puerto Rico, it was estimated that our original CDC-AGO resulted in a 43 percent reduction in the abundance of parous and gravid *Ae. aegypti*. To improve the potential of the trap as a vector control and surveillance tool, we evaluated several modifications to the design in competitive assays performed under laboratory and semi-natural conditions. The following changes to the trap significantly increased capture efficiency: increasing the size of the trap entrance, altering the color of trap components, and increasing the volume/surface area of the aqueous bait. The use of olfactory baits other than hay infusion (eg. synthetic baits, other organic substrates) did not improve trap performance. In a field study, mean (± SE) numbers of adult *Ae. aegypti* females captured per trap per day were 1.16 ± 0.05 in the modified CDC-AGO (max. collection = 6.75), and 0.36 ± 0.02 in our original CDC-AGO (max. collection = 2.67). Semiweekly collections of *Ae. aegypti* females in the modified trap were more significantly correlated with cumulative rainfall 12 - 25 days prior to sampling than in the original CDC-AGO or collections of eggs in standard ovijars. In a second field test, average semiweekly capture rates were as high as 3.3 *Ae. aegypti* females per trap per day in the modified CDC-AGO. The modified CDC-AGO was highly attractive to gravid *Ae. aegypti* females for up to 18 weeks without need for maintenance.

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THE IMPACT OF ANTI-VECTOR INTERVENTIONS ON THE EFFECTIVE POPULATION SIZE OF MALARIA MOSQUITOES

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Malaria vector control in the form of indoor residual spraying (IRS) and insecticide treated net (ITN) distribution has the ability to significantly impact malaria transmission. Although a reduction in malaria mosquito abundance has been reported following vector control, we know little about the extent to which IRS and ITN distribution is capable of reducing the effective size (N_e) of mosquito populations. A nationwide malaria control program has been implemented in Equatorial Guinea under the Bioko Island Malaria Control Project and the Equatorial Guinea Malaria Control Initiative. Anti-vector interventions under these programs consist of IRS and ITN distribution. We examined the impact of these interventions on the effective size of three species of malaria mosquitoes in several locations on Bioko Island and the Equatorial Guinea mainland. Microsatellite data were collected for several time points from two populations on Bioko Island, and 5 populations on the mainland. These populations include four *Anopheles gambiae* populations, two *An. melas* populations and a single *An. moucheti* population. These data were analyzed using a coalescent-based Approximate Bayesian Computation which allows a comparison of various demographic models. A demographic model describing a recent reduction in effective population size provided the best fit for the populations analyzed. Both IRS and ITN distribution had a substantial impact on the effective size of *An. gambiae* populations, reducing effective population size over 5- fold in the populations examined. Additionally, the timing of this N_e reduction matches well the implementation of vector control. These results show that IRS and ITN have a remarkably large impact on the size of *Anopheles gambiae* populations and that much of their effectiveness can be ascribed to decreases in mosquito populations, rather than a reduction in contact between mosquitoes and their human hosts.

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EFFECTIVENESS OF VARIOUS CHEMICAL COMPONENTS AS ATTRACTANTS TO PHLEBOTOMINE SAND FLIES IN TWO DIFFERENT ECOLOGICAL AREAS OF PERU

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Sand flies are small bloodsucking flies in the family Phlebotominae (Diptera: Psychodidae) that transmit the pathogens of human diseases; e.g.,leishmaniasis, and bartonellosis. In the Peruvian Andes, cutaneous leishmaniasis and bartonellosis are endemic diseases and in some areas (Madre de Dios) of the Amazon Jungle, leishmaniasis is an endemic disease. Surveillance of the local sand fly fauna is essential to establish and maintain effective sand fly control programs that will then decrease human disease incidence. Sand flies are most effectively captured using human-landing methods, but there are many ethical concerns regarding this method. Alternatives to human-landing methods are: use of CDC light traps, and mouth aspiration of sand flies from resting sites in animal refuges or Shannon traps. However, these methods are time- and labor-intensive, and yield small quantities. In addition, capture of sand flies using the CDC light trap requires $C0_2$ (generated by dry ice or liquid nitrogen), which can be very difficult and expensive to obtain and transport to remote locations. For these reasons, it is imperative to find more efficient attractants that will attract *Lutzomyia* in quantities that will permit evaluation of population differences in abundance and diversity in time and space. The objective of this study was to compare, in two different ecological areas, the efficacy of CDC light traps using different combinations of lactic acid, octenol, benzaldehyde, and 4-methyl-2-pentanone with and without a $C0_2$ generator (yeast+sugar+water). We collected 2,755 sand flies in the Amazon Jungle area and 3,050 in the Andean area; we evaluated the difference between each treatment to determine which combinations enhanced the sand Oy capture-rates in each trap. According to these results, there is no statistical difference between treatments.

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BEHAVIORAL RESPONSES OF *AEDES AEGYPTI* USING EXPERIMENTAL HUTS IN AN URBAN ROWHOUSE DESIGN IN IQUITOS, PERU

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Dengue, transmitted by *Aedes aegypti,* is still one of the most impo11ant viral diseases worldwide. Due to the lack of an effective vaccine and treatment, mosquito control plays a vital role in the prevention of the disease. Today dengue control is based on reduction of adult vector populations using chemicals at toxic doses; however, insecticide resistance, environmental concerns. and adverse health effects are threatening the efficacy of this approach. Behavioral effects of insecticides at sub-lethal doses are being discussed and considered as one possible alternative. These effects include spatial repellency where adult mosquitoes are discouraged from entering a treated space thereby reducing human-vector contaand the probability of pathogen transmission.This study, conducted as part of a larger research program to field-validate a Push-Pull strategy to reduce *Ae. aegypti* inside homes, quantified spatial repellency effects under field conditions using a mark-release-recapture experimental hut design. The uniqueness of our approach is that the five experimental huts share an adjoining wall with another hut and have open eave gaps thereby creating a continuum of indoor space. Such a "rowhouse" configuration is typical of Iquitos and other urban environments where dengue is endemic. This complex environment allows us to measure intensity of distance effects of a spatial repellent tool when varied numbers of huts arc treated with the intervention. We report on the reduction of indoor *Ae. aegypti* densities within each of the individual hut "cells" using several spatial repellent chemicals and doses. This information will help to determine required coverage rates of a spatial repellent intervention and optimize the "push" component of the overall Push-Pull strategy in preparation for a pilot field trial in local homes.

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A COMMUNITY-WIDE REPELLENT TRIAL: EVALUATING THE EFFICACY AND USER-ACCEPTANCE OF LOW-COST MOSQUITO REPELLENT IN GHANA

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Commercial repellents have been available in developed countries for decades, but their application to infectious disease problems in less developed countries has been frustrated by doubts about their efficacy, affordability, and user-acceptance in reducing vector-borne diseases. We evaluated the efficacy, user-acceptance and epidemiological efficacy of

NO MAS (NM), a water-based formulation whose active ingredients are PMD (para-menthane-diol) and lemongrass oil against local anopheline vectors of malaria in rural Ghana.The field test was carried out in Korania, a community within the Kassena Nankana District of Northern Ghana. A total of 64 man-nights captured 10% (576) *Anopheles* mosquitoes in the treatment arm (NM users) and 90% (5486) in the control arm (non-NM users). The biting pressure of *Anopheles* on an unprotected individual in the area was 86 bites/man/night compared to 9 bites/ man/night when the person uses NM repellent. The average percentage level of protection (efficacy) of NM repellent in the community was 89.6%. After 37,710 user- days, a NM repellent user-acceptance rate of 96.7% was estimated for the community. The probability that members of the community at risk of vector borne infections can escape infection by using the repellent based intervention was calculated as *Fe*=0.997549 translating to about 0.002451% of malaria infections. In the absence of the repellent, the probability of escaping malaria infection was *Fe*=0.981256 translating to about 0.018744% of malaria infections.With a protection level of 90%, the relatively high epidemiological efficacy and the low cost of the repellent, when distributed and used en masse as in combination with ITNs and IRS in the country, it can offer a powerful synergy that can lead to the drastic reduction of malaria and lymphatic filariasis transmission in many rural poor communities in West Africa including Ghana.

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EFFECTS OF LONG-LASTING INSECTICIDE TREATED NETS IN A DIVERSE VECTOR ENVIRONMENT

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In Papua New Guinea (PNG) members of the Punctulatus Group, including *Anopheles punctulatus, An. koliensis, An. farauti* s.s., *An. farauti* 4 and *An. hinesorum* (formerly *An. farauti* 2), exhibit heterogeneities in distribution, biting behavior and malaria infection levels. The PNG National Department of Health recently launched a nationwide long-lasting insecticide-treated net (LLIN) program. This study aimed to evaluate the impact of the campaign on anopheline species density, composition, feeding behavior and malaria infectivity. Sentinel sites were chosen from 6 PNG provinces representing coastal, riparian, inland and highland regions. Entomological surveys were conducted one year prior to, and two years post-LLIN distribution. Host-seeking anophelines were collected by the landing catch method from 6pm to 6am (N=46,000). Adults were identified to morphospecies and confirmed by PCR-RFLP of the internal transcribed spacer 2 rDNA. Malaria infectivity was determined by circumsporozoite ELISA for *Plasmodium falciparum*, *P. vivax* 210 and *P. vivax* 247. A reduction in man-biting rates and *Plasmodium* infectivity was observed for each species and from each location in the first year following LLIN distribution. Most vectors exhibited a shift to earlier peak biting times. In villages with multiple vector species, a significant change in species composition was observed, with the earlier biters, members of the Farauti complex, dominating the collections. By the second year after LLIN distribution, man-biting rates increased, in some cases rivalling the pre-distribution levels. This shift in biting times will result in greater exposure to the vectors despite bednet usage and the changes in species composition may alter malaria transmission dynamics. The implementation of singular control strategies in areas with diverse vector communities requires careful consideration.

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ASSESSMENT OF MONKEYPOX KNOWLEDGE IN HEALTHCARE WORKERS FOLLOWING TRAINING - DEMOCRATIC REPUBLIC OF CONGO, FEBRUARY 2011

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Estimating the incidence of monkeypox in the Democratic Republic of Congo (DRC) has been difficult due to inconsistent reporting and a paucity of laboratory confirmation for suspected infections. To improve monkeypox surveillance we trained 59 healthcare workers from Tshuapa District, Equateur Province in monkeypox surveillance methods, clinical case recognition, and specimen collection. In order to evaluate the effectiveness of the training program, a survey of monkeypox-specific knowledge was conducted before and after training. In general, pre-training monkeypox knowledge was high, with an average score of 26/37, which increased by 11% post training. With respect to monkeypox symptoms, the greatest improvement was seen in the participants' ability to identify the following symptoms as being associated with monkeypox: deep seated lesions (39% vs. 93%; $p<0.0001$) and lymphadenopathy (47% vs. 89%; $p<0.0001$). After training, more participants were able to differentiate between clinical photos of monkeypox and varicella cases (44%, 93%; $p<0.0001$) and to identify vesicular fluid and crusts as the preferred specimens for clinical diagnosis of monkeypox (7% vs. 61%; $p<0.0001$). However, there was also an increase in the number of people spuriously identifying water as a mechanism for monkeypox transmission (12% vs. 29%; $p<0.02$). The reason for this increase is unclear. Future educational materials will be refined to better address deficiencies identified through this evaluation. The impact of this training on surveillance efficacy remains to be determined; however, during the 2 months after the training diagnostic specimens were submitted for 55% of reported cases, as opposed to 1% throughout 2010.

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THE SURVEY OF DISTRIBUTION CHARACTERISTICS OF MOSQUITOES AND MOSQUITO-BORNE ARBOVIRUSES IN NORTHEAST AND SOME OTHER AREAS OF YUNNAN PROVINCE

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To investigate the distribution characteristics of mosquitoes and mosquito-borne arboviruses in northeast and some other areas of Yunnan province, and to provide scientific basis for prevention and control of the arbovirus disease. Mosquitoes were collected in 6 counties of northeast and some other areas of Yunnan province in 2009. After classification and determination, all mosquitoes were used to viruses isolation. Positive isolates were identified by SDS-PAGE and RT-PCR, then sequenced and

phylogenetic analyzed. 4 genus (*Culex, Anopheles, Armigeres, Aedes*), 24 species, 18,562 mosquitoes were collected. *Cx. tritaeniorhynchus*, *An. sinensis* were main species in total, and their constituent ratios were respectively 58.37% (10,834/18,562) and 28.45% (5281/18,562). 15 strains of viruses were isolated from mosquito pools. By RT-PCR and phylogenetic analysis, 2 strains isolated from *Cx. tritaeniorhynchus* were identified as Japanese encephalitis virus (JEV, Genotypel). 1 strain isolated from *An. sinensis* was identified as Bannna virus. 12 strains were identified as *Cx. pipens pallens* densovirus (CppDNV), 9 strains of them were isolated from *Cx. tritaeniorhynchus* and 3 strains of them were isolated from *Anopheles sinensis*. *Cx. tritaeniorhynchus* and *An. sinensis* were the predominant species in the investigated areas. Japanese encephalitis virus, Bannna virus and CppDNV were isolated here. It was the first time that Japanese encephalitis virus had been isolated in northeast of Yunnan, China.

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CRIMEAN CONGO HEMORRHAGIC FEVER SURVEILLANCE IN KAZAKHSTAN, 2009-2010

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Crimean Congo Hemorrhagic Fever (CCHF) virus is a tick-borne pathogen that causes hemorrhagic fever symptoms with high fatality in hospitalized patients. While tick bites are an important means of transmission, little population-based data has been collected concerning tick bites and CCHF incidence. CCHF is endemic in Kazakhstan, with most cases occurring in the Southern Kazakhstan Oblast (SKO) region. Surveillance activities in this region included reporting of suspect and confirmed cases, and a registry of reported tick bites. We analyzed surveillance data for CCHF in Southern Kazakhstan, in order to better understand disease dynamics and evaluate tick bite reports as an indicator of CCHF risk. Line lists of CCHF case-patients were reviewed. Weekly summaries of tick bites reported in SKO during spring and summer of 2009-2010 were obtained, and the spatial and temporal incidence of tick bites and CCHF were compared. Twenty-two CCHF cases were reported in 2009 and 17 in 2010. Of the reported CCHF patients, 38% reported livestock exposures, 33% reported known tick exposures, 15% had nosocomial exposures and 14% had no risk factors identified. Weekly total reported tick bites in 2009 and 2010 correlated significantly with weekly CCHF occurrence (2009 $r = 0.58$, $p = 0.002$; 2010 $r = 0.60$, $p < 0.001$). Additionally, the incidence of tick bites was significantly higher in municipalities reporting CCHF cases than in those with no CCHF cases ($p = 0.01$). In conclusion, an analysis of CCHF surveillance data in Kazakhstan found a high number of reported tick bites, with spatial and temporal association between tick bites and CCHF cases. Public health measures should center on prevention of tick bites in people, increasing awareness of CCHF signs and symptoms in populations at risk of tick and livestock exposure, and adoption of infection control practices in the hospital setting.

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NOROVIRUS GASTROENTERITIS IN ECUADOR: DATA FROM A PILOT STUDY IN A RURAL DISTRICT

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Norovirus is the leading cause of both outbreaks and sporadic acute gastroenteritis in developed-countries. There are limited data on the epidemiology and burden of norovirus gastroenteritis in Latin America. We analyzed fecal samples for the presence of norovirus from children presenting with diarrhea in a rural District of Esmeraldas Province, Ecuador. The samples were from children aged 6-13 months in a surveillance cohort of 195 children nested within the ECUAVIDA birth cohort. A total of 190 stool samples from infants with diarrhea were analyzed by real-time reverse transcription-PCR to identify norovirus genogroup (G) I and II. Overall 43 (22.6%) samples tested positive for norovirus with 16 (8.4%) for norovirus GI and 27 (14.2%) for norovirus GII. The data suggest that norovirus infection is a significant cause of gastroenteritis in very young children in rural Ecuador.

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THE GERMAN ARBOVIRUS SURVEILLANCE AND MOSQUITO MONITORING PROGRAM, 2009 - 2010

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The aim of the program is to provide an early warning of the presence of arboviruses in Germany. The program compiles and analyses mosquito and arbovirus data collected over a number of successive years. This will provide a solid base to determine the underlying causes of the seasonal fluctuations in arbovirus activity and the relative abundance of the mosquito vector species. This information can then be used as a basis for vector control programs. During 2009 and 2010 we monitored mosquito vector populations and undertook surveillance of arbovirus activity mostly in South West Germany. Approximately 90,000 mosquitoes were captured and assayed for the presence of arboviruses. In 2009, Sindbis virus (SINV) and Batai virus (BATV) were isolated from *Culex* spp. and *Anopheles maculipennis s.l.*, respectively. The highest SINV infection rate (4.9) in the *Culex* mosquitoes was in the beginning of July. Phylogenetic analysis of the German SINV strains linked them with Swedish SINV strains, the causative agent of Ockelbo disease in humans. Analysis of partial S, M, and L segments of the German BATV strain showed that the sequences from all three segments were most closely related to BATV, indicating that the virus has not undergone reassortment. In contrast, only Usutu virus (USUV) was isolated in 2010 from *Culex* spp. and demonstrated to be related to USUV strains circulating in Austria and Italy. Further studies have to be conducted to estimate the veterinary and medical importance of SINV, BATV and USUV in the affected areas.

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IMMUNE FUNCTION AND MICRONUTRIENT STATUS OF PREGNANT WOMEN INFECTED BY HEPATITIS E VIRUS IN BANGLADESH BETWEEN 2001 AND 2010

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Hepatitis E virus (HEV) is the leading cause of acute viral hepatitis globally and results in severe morbidity and mortality in pregnant women. There is a paucity of longitudinal data examining the incidence and disease rate of HEV in cohorts of pregnancy in endemic areas. We studied serial sera collected within two prospective cohorts totaling 110,473 incident pregnancies enrolled large randomized trials in rural northwestern Bangladesh, between 2001 - 2007 (cohort A) and 2007 - 2010 (cohort B). An NIH research immunoassay was used to identify anti-HEV IgG status in early pregnancy, late pregnancy and 3 month postpartum venous blood specimens, drawn on a subsample of the larger cohorts. Of the 1,127 specimens available for testing in cohort A, 72 were anti-HEV seropositive at baseline, indicating a seroprevalence of ~6.4%. During

this period, 63 women were identified as potential seroconverters, suggesting an incidence rate of ~56 infections per 1000 person-years. In the more recent cohort B, 1100 were available for testing, revealing a ~6.1% seroprevalence in anti-HEV IgG at early pregnancy. Within this cohort, 40 women were identified as putative seroconverters, an incidence rate of 46 infections per 1000 person-years. Between the 2001 to 2006 cohort and the 2008 to 2010 cohort, the incidence of intrapartum HEV infections seems to be declining in rural Bangladesh, possibly reflective of improved sanitation. Cytokine and micronutrient analysis of the 2008 to 2010 cohort is ongoing to characterize the immunopathology of HEV infection. In the cohort A, 4 pregnant seroconverters with high antibody titers were evaluated for cytokine profiles, revealing elevated levels of pro-inflammatory cytokines compared to unifected controls and women who were seropositive at baseline. Treg-associated IL-10 levels also seem to be elevated in HEV-infected cases. Although no pregnancy-related mortality was observed in this nested cohort, analysis is ongoing to assess whether any sign of immune dysregulation or immune response inconsistent with late pregnancy is evident. Initial data suggests seroconverters seem to have lower baseline serum Zinc levels than their non-infected counterparts. Vitamin D and Copper (Cu) levels were also lower, although not statistically different. This data also seeks to elucidate population-based rates of HEV disease:infection ratios within a non-epidemic context, where this pathogen is ubiquitous.

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DIFFERENTIAL REPLICATION OF EPIZOOTIC VERSUS ENZOOTIC SUBTYPE IE VENEZUELAN EQUINE ENCEPHALITIS VIRUSES IN EQUIDS

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The etiologic agents during major Venezuelan equine encephalitis virus (VEEV) outbreaks are associated with subtypes IAB and IC, while subtype IE strains are generally considered enzootic, equine-avirulent, and incapable of exploiting horses as amplification hosts. However, Mexican epizootics on the Pacific Coast were shown to originate from equine-virulent subtype IE VEEVs. To determine whether the virulence of these subtype IE VEEVs correlated with the development of viremia, equine pathogenesis studies were performed, in which groups of horses were inoculated subcutaneously with one of three VEEV strains: 1) MX01-32, an unpassaged subtype IE strain genetically and geographically related to the Mexican outbreaks, 2) 68U201, a related enzootic subtype IE VEEV from Guatemala, and 3) 3908, an epizootic subtype IC VEEV strain from the last major equine-amplified epidemic. MX01-32-infected horses developed a febrile response and viremia that was comparable in titer to and earlier than that induced by 3908. To determine whether these *in vivo* findings could be correlated to the replication kinetics of these viruses *in vitro*, equine peripheral blood monocyte cultures were infected with either: 1) vesicular stomatitis virus (VSV) [a positive control virus known to replicate efficiently in equine monocytes], 2) 3908, 3) ZPC738 (an enzootic subtype ID VEEV), 4) 68U201, or 5) MX01-32. Culture supernatants were collected and titered by plaque assay. The Mexican strain of subtype IE VEEV (MX01-32) replicated to a higher titer than the enzootic Guatemalan strain (68U201), corroborating evidence that the virulence of epizootic subtype IE viruses depends on virus replication. To quantify the relative number of infected monocytes, indirect fluorescent antibody (IFA) assays were performed, which showed that, regardless of VEEV subtype, there were relatively few infected cells compared to the total number of cells in culture. These results suggest an intrinsic mechanism of modulating viral replication in equids, which may be a critical factor for the successful development of intervention strategies that protect human populations from future outbreaks.

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MULTIPLEX MICROSPHERE IMMUNOASSAYS FOR THE DETECTION OF IGM AND IGG TO ARBOVIRUSES

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A variety of techniques have been developed over the past 40 years for the serodiagnosis of arboviruses. These include immunofluorescence assay, complement fixation test, hemagglutination inhibition assay, plaque reduction neutralization test, and IgM and IgG enzyme-linked immunosorbent assays (ELISAs). The most recent addition to the menu of tests is the microsphere assay (MIA) which uses the Luminex platform. The use of combined serologic testing data is currently the method of choice for laboratory diagnosis of arboviruses. MIAs have been used as screening tools for arboviruses over the past 5 years. A number of US State and government labs including the CDC have used a duplex IgM test for West Nile (WN) and St. Louis encephalitis (SLE) viruses, and have participated in proficiency testing using this method. The speed and ease of use of this platform have made these tests attractive for expansion to other arboviruses, where viral antigens of interest can be incorporated into the testing battery as needed. The creation of IgM and IgG multiplex MIAs allow for a comprehensive array of arboviral infections to be tested for concurrently. Here we report the development of multiplex assays for IgM and IgG to 6 flaviviruses, 6 alphaviruses, and 1 bunyavirus of human importance, incorporating validation results for its practical use in geographic batteries. Internal test controls were included in the assays to boost confidence in the results. Samples from previous diagnostic submissions were used to generate MIA data, which were compared to those of IgM and IgG ELISAs and to the overall laboratory diagnoses for the patients. Six classification methods were compared to determine which performed best for this application, with linear kernel support vector machines proving to be the best for the geographic batteries. The analyses will be presented.

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ORTHOBUNYAVIRUSES ARE A COMMON CAUSE OF INFECTION IN DOMESTIC ANIMALS IN THE YUCATAN PENINSULA OF MEXICO

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A serological investigation was performed to determine the seroprevalence of various orthobunyaviruses in domestic animals in the Yucatan Peninsula of Mexico. The study was performed using an archived collection of sera taken from 256 domestic animals (182 horses, 31 sheep, 1 dog, 37 chickens and 5 turkeys) at multiple study sites in the Yucatan Peninsula between September 2007 and October 2008. All sera were initially screened at a single dilution (1:20) by plaque reduction neutralization test (PRNT) using five orthobunyaviruses: Cache Valley virus (CVV), Maguari virus (MAGV), South River virus (SORV), Kairi virus (KRIV) and Wyeomyia virus (WYOV). If neutralizing antibodies were detected, the sample was further diluted and subsequent PRNTs were performed to determine the end-point $PRNT_{90}$ titer. Remarkably, antibodies to orthobunyaviruses were detected in most of the sera tested. Of the 182 horses analyzed, 83 (46%) were seropositive for CVV, 18 (10%) were seropositive for MAGV, 2 (1%) were seropositive for SORV, 64 (35%) had antibodies to an undetermined orthobunyavirus and 15 (8%) were negative. Of the 31 sheep analyzed, 8 (26%) were seropositive for CVV, 4 (13%) were seropositive for SORV, 15 (48%) had antibodies to an undetermined orthobunyavirus and 4 (13%) were negative. The single dog analyzed in this study was seropositive for SORV. Additionally, 4 (11%) chickens had antibodies to an undetermined

orthobunyavirus and 1 (20%) turkey was seropositive for CVV. These data indicate that orthobunyaviruses are a common cause of infection in some species of domestic animals in the Yucatan Peninsula of Mexico.

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ALPHAVIRUS INFECTION AMONG PEDIATRIC ENDEMIC BURKITT'S LYMPHOMA IN KENYA

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Arboviral infections have been implicated as precursors to the onset of lymphoma. In regions of equatorial Africa, endemic Burkitt's lymphoma (eBL) and arboviral infection overlap in both geography and demography. The most prevalent childhood cancer in Kenya is eBL. Alphaviruses, such as chikungunya virus (CHIKV), are also common in Kenya. During a previous arboviral serosurvey conducted in western Kenya (N=122, median age=8.59, mean age=9.04), 20% of children were positive for CHIKV-specific IgG by immunofluorescence (IFA) testing (N=24, median age=9.62, mean age=9.55). A sample of pediatric eBL-positive Kenyan children was tested for CHIKV IgG by IFA (N=48, median age=6, mean age=6.62). eBL samples were more likely to be CHIKV IgG positive (35% vs. 20%, p =0.0453). Of the CHIKV positive eBL samples, 59% were boys and 41% were girls. Gender was not associated with CHIKV-status (p=0.7611), nor was prior malaria treatment (p=0.1765). Curiously, eBL children who were CHIKV seropositive had a higher survival rate (76% vs. 42%, p=0.0339). After controlling for tumor site, CHIKV was still associated with eBL survival. Given these preliminary data, chikungunya virus exposure is associated with endemic Burkitt's lymphoma among children in this study area. Further investigation of the effects of alphaviruses and other arboviruses on eBL incidence, prevalence and outcomes may be warranted given the high prevalence of both arboviruses and eBL in the region.

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VIRAL HEMORRHAGIC FEVER SURVEILLANCE IN UGANDA (2010-2011)

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Uganda is endemic for viral hemorrhagic fevers (VHFs), including Ebola, Marburg, Rift Valley Fever (RVF) and Crimean Congo Hemorrhagic Fever (CCHF) viruses. In order to enhance the ability to identify and rapidly test for VHF's, in July 2010 the Viral Special Pathogens Branch (VSPB), CDC, the Uganda Virus Research Institute (UVRI), and the Ministry of Health (MOH) established a National VHF surveillance program. Since July 2010, VSPB Uganda has established 6 sentinel VHF surveillance sites and trained 28 clinical and laboratory staff on the identification, reporting, infection control, and clinical sample collection procedures for suspect VHF cases. The laboratory has also processed more than 70 clinical samples for suspect VHF. Notably, the program was alerted in early October, 2010 of an "unknown illness" in Northern Uganda and performed rule-out testing on 55 suspect cases. Acute diagnostic testing for Ebola hemorrhagic fever was performed by PCR and antigen detection and for Marburg hemorrhagic fever by PCR on all clinical samples, indicating that neither Ebola nor Marburg virus was the etiologic agent. Among acute samples with an known onset date, the median time from symptom onset to sample collection was 3 days, and median time for all acute samples tested, from either collection or case report to diagnostic rule-out for EHF and MHF was 6 days (by PCR), 13 days (by serology). A subset of 17 samples was subsequently sent to CDC, Atlanta for further testing for viral hemorrhagic fever; all were demonstrated negative for Rift Valley Fever (by PCR and IgM ELISA) and Crimean Congo hemorrhagic fever (by PCR). Next generation sequencing (NGS) was employed to detect pathogen genomic material amplified from patient serum for Yellow fever virus (YFV) in one of four patient sera tested; the first YFV case detected in Uganda since 1964. The Uganda VHF surveillance program has the ability to rule-out commonly suspected viral hemorrhagic fevers in a timely manner and continues to receive suspect VHF cases reports and perform diagnostic testing to rule out VHFs.

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TRANSFORMING GROWTH FACTOR-β AND INTERLEUKIN-10 ALTER HANTAVIRUS CARDIOPULMONARY SYNDROME DISEASE SEVERITY

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Sin Nombre virus (SNV) was first identified in 1993 in the Four Corners region of North America as an etiologic agent of hantavirus cardiopulmonary syndrome (HCPS). Infection is associated with high levels of inflammatory cytokine staining in human pulmonary autopsy specimens, suggesting HCPS is an immunopathology. The reservoir of SNV is the deer mouse (Peromyscus maniculatus), which develops persistent infection without pathology. Experimental data showed increased expression of transforming growth factor beta-1 (TGFβ1) in virus-specific helper T cells from these animals. The Syrian golden hamster (Mesocricetus auratus) has been used as an HCPS model with Maporal virus (MAPV). Our hypothesis predicted use of TGFβ1 or interleukin-10 (IL-10) as a therapeutic agent would attenuate disease severity. Gene expression in both lung and spleen suggested an innate immune response. Spleens had increased chemokine expression of IL-12 (both p35 and p40 subunits), as well as p27 and Eif2ak2 that indicated an attempt by cells to limit viral protein synthesis and cell division. Lungs had increased expression of CXCL10, ICAM-1, PECAM and VEGF which also suggest attraction of leukocytes. By day 10 there was an increase in adaptive immune cytokines in both spleens and lungs. Spleens had increased expression of IL-6 and IL-21 genes, suggestive of a CD4+ T cell response. Lungs had a notable increase in MHC-II gene expression. The administration of TGFβ1 appeared to suppress expression of IL-12 in spleens and MHC-II in lungs. Hamsters infected with MAPV and treated with TGFβ1 had decreased lung congestion and pleural fluid, although no significant attenuation of disease was observed. Administration of IL-10 resulted in increased lesion score and no suppression of gene expression.

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MYELOID CELL RELA PROMOTES ROSS RIVER VIRUS-INDUCED MUSCULOSKELETAL DISEASE

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Ross River virus (RRV) and chikungunya virus (CHIKV) are mosquito-transmitted alphaviruses that cause debilitating rheumatic disease in humans. Studies in humans and animal models suggest that macrophages have critical pathogenic and protective roles in alphavirus-induced rheumatic disease, but specific macrophage effector mechanisms that mediate these effects have not been defined. Nuclear factor-κB (NF-κB) is a transcription factor that regulates the activation of myeloid cells during inflammatory reactions, and inhibitors of NF-κB have been shown to reduce the severity of RRV-induced disease. To investigate the role of myeloid cell NF-κB activity in the pathogenesis of RRV/CHIKV infection, we bred mice to delete the canonical NF-κB subunit RelA (p65) specifically from myeloid cells (LysMCre;RelAflox). Following

RRV infection, LysMCre;RelAflox mice had less severe altered gait and deficits in gripping ability, as well as improved weight gain compared to control mice. These findings suggest that RelA activity in myeloid cells promotes RRV-induced rheumatic disease. Interestingly, despite less severe rheumatic disease signs, LysMCre;RelAflox mice had higher viral loads in tissues. Taken together, our data suggest that RelA regulates myeloid cell effector functions that mediate tissue injury and/or virus control during RRV infection. We propose that further studies in this model may allow us to define specific effector mechanisms by which myeloid cells mediate protection or pathology following RRV/CHIKV infection, leading to a better understanding of the pathogenesis of these infections and aiding in the rational design of targeted therapeutics.

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LIVE BIRD MARKET ENVIRONMENTAL SAMPLING: A TOOL FOR POULTRY INFLUENZA SURVEILLANCE

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Since 2007, Bangladesh has annually reported influenza A/H5 poultry outbreaks during January-April with sporadic infections among humans. We conducted live bird market surveillance to identify circulating influenza viruses in poultry. From May 2009 to March 2011 we collected environmental samples every month from three rural and eight urban live bird markets in Bangladesh. The weekly rural markets were distributed across the country and were selected based on their higher poultry population density. Eight urban markets were in Dhaka city, where the dealers daily brought poultry from different districts. Once a month, we swabbed poultry cages, feed and water trays, and fecal material from the poultry stalls of the birds and collected 10 swabs as convenience samples of multiple sites and pooled them into one environmental sample per market. We conducted real-time reverse transcription polymerase chain reaction (rRT-PCR) for identifying influenza A viruses and H5 sub-type. We collected 213 pooled environmental samples from the live bird markets. Of the 72 environmental specimens collected from rural markets, 32 (44%) were rRT-PCR positive for influenza A viruses and of these, two (3%) were positive for H5 virus. Of the 141 environmental specimens collected from eight urban live bird markets, 100 (71%) were rRT-PCR positive for influenza A viruses and of these, 49 (35%) were H5 virus positive. The environmental samples collected from the urban live bird markets were three times more likely to be influenza A positive compared with the rural samples (p=<0.001). The majority (n=47, 96%) of the influenza A/H5 virus positives in Dhaka markets were identified during October and March, the cooler months (mean temperature: 23 degree C; range: 20-27 degree C) of the year in Bangladesh. During the same time period, 171 (89%) poultry outbreaks were reported nationally. In conclusion, we frequently identified influenza A/H5 in the urban live bird markets concurrent with seasonal outbreaks reported throughout Bangladesh. Urban live bird markets serve as collection points of poultry from throughout the country may act as sentinels for circulating influenza viruses. Live-bird markets may be high-risk sites for harboring influenza viruses and prime sites for interventions aimed at preventing transmission in poultry.

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HEPATITIS E VIRUS DETECTION AND CHARACTERIZATION IN SEWAGE FROM VELLORE, SOUTH INDIA

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Hepatitis E virus (HEV) is enterically transmitted and causes an acute, self-limiting hepatitis. In developing countries, HEV is endemic. The virus is hyperendemic in India leading to frequent water-borne epidemics and high rates of sporadic acute hepatitis. Fecal shedding of HEV from both humans and animals maintains the virus in sewage. Since sewage systems are important points to monitor enteric pathogens transmitted through water, we carried out a monthly sampling and testing for HEV in sewage. For this study to detect and characterize HEV in sewage and compare its frequency and seasonal pattern with another enteric pathogen, rotavirus and HEV were investigated in sewage from Vellore, a city in the state of Tamil Nadu in South India. From November 2009 to October 2010, 12 sewage samples were collected each month from the major sewage outlets, where the city's untreated sewage is discharged. A total of 144 raw sewage were tested for HEV RNA and rotavirus. Viral particles in Sewage were pelleted using ultra-centrifugation based concentration method. The total RNA extracted was subjected to polymerase chain reaction using specific primers for HEV and rotavirus. HEV strains isolated from sewage were sequenced. The overall prevalence of HEV RNA in sewage was 55.5% and that of rotavirus was 77%. HEV RNA was identified more often during the summer (81.2%) compared to the monsoon (14.5%) (P <0.001), while rotavirus was found more often in winter (97.9%) than during the monsoon (50%) (P <0.001). All the HEV strains isolated from sewage belonged to genotype 1. They were genetically closely related to HEV strains from Swedish nationals who were infected while travelling in India and HEV strains implicated in a large outbreak in Nellore, South India. The frequency of HEV in sewage from Vellore, South India was higher than reports from other parts of India. HEV strains in sewage from Vellore are of human, not animal, origin. This study underscores the need for preventive measures to protect drinking water from sewage contamination, particularly in the summer.

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RTS,S/AS01 MALARIA VACCINE CANDIDATE PHASE III EVALUATION: EFFICACY AGAINST CLINICAL MALARIA IN AFRICAN CHILDREN 5-17 MONTHS OF AGE

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In phase II clinical trials, the RTS,S/AS01 malaria vaccine candidate provided protection against malaria in African children living in malaria endemic regions. Recent results showed 39% and 42% vaccine efficacy (VE) against first episode and all episodes clinical malaria respectively, over 12 months. Investigators are now conducting a large multi-center phase III randomized, double-blind, trial. The trial has enrolled 15,460 children in two age categories, 5-17 months, and 6-12 weeks. We will present results from the primary analysis on 6000 children aged 5-17 months, during 12 months following vaccination. We will focus on VE measures using primary and secondary case definitions of clinical malaria. This ongoing phase III randomized, double-blind, controlled trial is being conducted at 11 sites in 7 African countries, representing diverse malaria transmission settings. Children aged 5-17 months whose parents provided informed consent were randomized 2:1 to receive the RTS,S/AS01 candidate malaria vaccine or comparator (rabies) vaccine, administered monthly for 3 doses. Clinical malaria episodes were captured by passive case detection. The primary case definition was *P. falciparum* parasitemia >5000 parasites/μL in an unwell child brought to a study clinic with temperature ≥37.5°C, or a case meeting a standardized primary case definition of severe malaria disease. Secondary case definitions differ in parasite density thresholds: >0, >500, >20,000 parasites/μL.VE will be assessed using Cox regression models (first episodes) and negative-binomial regression (multiple episodes). Anti-CS antibody titers were measured with a validated ELISA test at enrollment and one month post vaccine dose-3. VE against the first or only episode of clinical malaria and against multiple episodes of clinical malaria will be presented for all parasite density thresholds. Primary analysis will be according to protocol (12 months post-dose 3). An intention to treat analysis (14 months post-dose 1) will also be presented. Anti-CS antibody response at 1 month post dose-3 will be presented.

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RTS,S/AS01 MALARIA VACCINE CANDIDATE PHASE III EVALUATION: EFFICACY AGAINST SEVERE MALARIA DISEASE

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The RTS,S/AS01 candidate vaccine is being developed with the aim of reducing the burden of malaria including the approximately 800,000 deaths that occur each year, mainly in African infants and young children. Vaccine efficacy against the most severe forms of disease will be an important driver of the vaccine implementation decision process. The protective efficacy of RTS,S/AS01 against severe malaria is currently being evaluated in a multicenter Phase 3, randomized, controlled, double blind trial in children aged 5 to 17 months or 6 to 12 weeks old at first vaccination, across 11 research sites in 7 African countries. A standardized clinical algorithm for evaluation of sick children is being used to capture severe malaria cases in children presenting to clinical facilities. The primary endpoint definition of severe malaria includes a positive *P. falciparum* parasitemia over 5000/µL, specific clinical and laboratory markers associated with a risk of an adverse outcome and the absence of important co-morbidities. Vaccine efficacy against severe malaria will also be evaluated using additional secondary case definitions which include other parasitemia thresholds or allowing for inclusion of cases presenting with comorbidities. Here, we present an initial analysis of vaccine efficacy against severe malaria which will be based on an analysis of the entire trial population (ATP cohort for efficacy) up to the point when approximately 250 cases have been accumulated, as well as upon 1 year follow up after the primary vaccination schedule in children in the 5-17 months age group. Findings from the Phase III RTS,S/AS01 trial will provide key evidence on whether the vaccine can play a role in reducing the risk of severe malaria and associated co-morbidities in the target population.

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STRAIN-SPECIFIC *PLASMODIUM FALCIPARUM* GROWTH INHIBITION AMONG MALIAN CHILDREN IMMUNIZED WITH THE FMP2.1/AS02A VACCINE

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Additional authors: Issa Diarra, Amadou Niangaly, Amagana Dolo, Modibo Daou, Youssouf Tolo, Mady Sissoko, Shannon Takala-Harrison, Olivier Godeaux, Brent House, Jason W. Bennett. The blood stage malaria vaccine FMP2.1/AS02A, comprised of recombinant *Plasmodium falciparum* apical membrane antigen 1 (AMA1) and the adjuvant system AS02A, had strain-specific efficacy against clinical malaria caused by *P. falciparum* with AMA1 sequence corresponding to the vaccine strain 3D7. To evaluate a potential correlate of protection, we measured the ability of sera to inhibit the growth of 3D7 and FVO strains *in vitro* using high-throughput growth inhibition assay (GIA) testing. Sera from both AMA1 vaccine and control rabies vaccine groups were analyzed at baseline and eight subsequent time points over two malaria seasons. Baseline GIA against the vaccine strain 3D7 was similar in both groups, but a significantly higher proportion of AMA1 vaccinees had 3D7 GIA activity above a pre-determined threshold of 15% thirty days after the last vaccination (day 90) compared to the control group (49% vs 16%). From baseline to day 90 (corresponding to the start of the malaria season), 3D7 GIA in the AMA1 group increased 3.6-fold compared to 1.3-fold in the control group ($p<0.0001$). This increase was not associated with efficacy against all clinical malaria, but was associated with efficacy against clinical malaria with 3D7-type AMA1 sequence with respect to eight immunologically important amino acid residues in all participants ($p=0.01$). Baseline GIA against the FVO strain was also similar in both groups, but did not increase in either group. Analyses of GIA at additional time points are underway and may further elucidate the association of 3D7 GIA with protection. These results provide a potential immune correlate of strain-specific protection against clinical malaria for a blood stage vaccine, and will inform the development of more broadly protective next-generation malaria vaccines.

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PHASE 1/2A OPEN-LABEL DOSE SAFETY, REACTOGENICITY, IMMUNOGENICITY AND EFFICACY OF THE CANDIDATE *PLASMODIUM VIVAX* MALARIA PROTEIN 001 (VMP001) ADMINISTERED INTRAMUSCULARLY WITH GSK BIOLOGICALS' ADJUVANT SYSTEM AS01$_B$ IN HEALTHY MALARIA-NAÏVE ADULTS

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A vaccine to prevent infection and disease caused by *Plasmodium vivax* is needed both to reduce the morbidity caused by this parasite and as a key component in efforts to eradicate malaria worldwide. *Vivax* malaria protein 1 (VMP001) is a novel chimeric protein that incorporates the N- and C-terminal parts of the circumsporozoite (CS) protein and a truncated repeat region that contains repeat sequences from both the VK210 (type 1) and the VK247 (type 2) parasites. Following promising preclinical findings, we conducted a first-in-human Phase 1/2a vaccine study of VMP001 formulated in the GSK Adjuvant System AS01$_B$. The study was designed to incorporate dose-escalation, evaluating 3 antigen doses. A total of 30 volunteers were divided into 3 groups (10 in each group) and given 3 intramuscular injections at defined intervals of 15 µg, 30 µg, and 60 µg respectively, all in 500 µL of AS01$_B$ at each immunization. A *P. vivax* infected mosquito challenge was performed in 6 infectivity control volunteers and all volunteers from the 3 vaccine groups 14 days following the third immunization. The vaccine was shown to be safe and immunogenic; although it did not induce sterile protection, a small but consistent increase in pre-patent period was observed in some subjects. Volunteers who developed parasitemia were treated with chloroquine and primaquine as soon as parasites were identified on screening blood smears. This trial was the first testing of a *P. vivax* candidate vaccine in the clinic in conjunction with the *P. vivax* sporozoite challenge model. The ability to challenge vaccine recipients will accelerate the process of *P. vivax* vaccine development, allowing better selection of candidate vaccines for

advancement to field trials. The safety, reactogenicity, immunogenicity against VMP001, and efficacy of the vaccine against a *P. vivax* sporozoite challenge are reported.

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LACK OF PROTECTIVE EFFICACY OF AN ADENOVIRUS-VECTORED *PLASMODIUM FALCIPARUM* MALARIA VACCINE IN THE ABSENCE OF DNA PRIMING

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Malaria remains one of the world's major public health problems with a vaccine urgently needed. A multi-stage, multi-antigen prototype adenovirus (serotype 5)-vectored vaccine, designated NMRC-M3V-Ad-PfCA (AdCA), is under evaluation by the U.S. Military Malaria Vaccine Program (USMMVP). The vaccine is comprised of adenovectors encoding two malaria antigens: 1) circumsporozoite protein (CSP), expressed in sporozoite and early liver stages, and 2) apical membrane antigen 1 (AMA1), expressed in sporozoite, liver and erythrocytic stages. The USMMVP recently demonstrated that priming with a DNA vaccine encoding the same two malaria antigens followed by boosting with the AdCA vaccine sterilely protected 4 of 15 human volunteers against sporozoite challenge in association with strong CD8+ T cell-dependent interferon (IFN)-γ ELISpot responses. The current trial evaluated the protective efficacy of the AdCA vaccine given without DNA priming. A single dose of AdCA (1 x 10^{10} pu/antigen) was administered to 20 healthy, malaria naïve, Ad5 seronegative volunteers. Four weeks later 18 immunized and 6 unimmunized infectivity controls underwent homologous *Plasmodium falciparum* sporozoite challenge by the bites of mosquitoes. The AdCA vaccine was safe and well-tolerated. IFN- γ ELISpot responses were higher following AdCA in the absence of a DNA prime (CSP range 34-2508 sfc/10^6 PBMCs, geometric mean 236; AMA 1 range 399-4456 sfc/10^6 PBMCs, geometric mean 1102) than when the prime had been given in the previous trial (CSP range 5-375 sfc/10^6 PBMCs, geometric mean 43; AMA1 range 14-1165 sfc/10^6 PBMCs, geometric mean 177). However, all challenged volunteers became parasitemic with no significant delay to patency in the immunized compared with the control group. Although the AdCA vaccine administered alone stimulates quantitatively superior ELISpot responses against whole proteins than when following DNA priming, it does not confer sterile protection. This suggests a qualitative difference in the responses. The nature of the protective T cell response requires further investigation.

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LONGEVITY AND COMPOSITION OF CELLULAR IMMUNE RESPONSES FOLLOWING EXPERIMENTAL *PLASMODIUM FALCIPARUM* MALARIA INFECTION IN HUMANS

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Cellular responses to *Plasmodium falciparum* parasites, in particular interferon-gamma (IFNγ) production, play an important role in anti-malarial immunity. However, clinical immunity to malaria develops slowly amongst naturally exposed populations, dynamics of cellular responses in relation to exposure are difficult to study and data about the persistence of those responses are controversial. Here we assessed the longevity and composition of cellular immune responses following experimental malaria infection in human volunteers. We conducted longitudinal studies of cellular immunological responses to sporozoite (*Pf*Spz) and blood-stage (*Pf*RBC) malaria parasites in naïve human volunteers undergoing single or multiple experimental *P. falciparum* infections under highly controlled conditions. We show that induced cellular responses to both *Pf*Spz and *Pf*RBC remain present up to 14 months after even a single malaria episode. Remarkably, not only 'adaptive' but also 'innate' lymphocyte subsets contribute to the increased IFNγ response, including αβT cells, γδT cells and NK cells. The majority of responding T-lymphocytes express an effector memory phenotype both early and late post-infection and CD4+ cells outnumber CD8+ cells. We established that both γδT cells and αβT cells independently contribute to immunological memory. Finally, we demonstrate that malaria infection induces and maintains notable pluripotent (IFNγ+IL-2+) effector memory responses against both *Pf*RBC and *Pf*Spz, found previously to be associated with protection. These data demonstrate that cellular responses induced by infected mosquito bites can be readily induced and are long-lived with a continued interdependence between adaptive and (semi-)innate lymphocyte subsets.

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ADAPTIVE CLINICAL TRIALS OF THREE PFSPZ PRODUCTS FOR DEVELOPMENT OF A WHOLE SPOROZOITE VACCINE THAT PREVENTS *PLASMODIUM FALCIPARUM* INFECTION, DISEASE AND TRANSMISSION

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An ideal, single stage vaccine for elimination of *Plasmodium falciparum* (Pf) would prevent infection at the pre-erythrocytic stage of the life cycle, thereby preventing all Pf-caused disease and Pf transmission. The only approach to immunization that induces > 90% protection against infection that is sustained for at least 10-28 months, is immunization by mosquito bite with whole Pf sporozoites (SPZ) of two types. The first, radiation-attenuated PfSPZ, invades hepatoctyes and expresses new proteins, but cannot replicate. The second type fully develops in hepatocytes, producing tens of thousands of merozoites that invade erythrocytes, but cannot fully develop within erythrocytes, because the parasites are killed by chloroquine taken by during immunization. Sanaria was founded to develop PfSPZ vaccines. The 1st vaccine is based on radiation attenuated PfSPZ. The first task accomplished was production of PfSPZ that met regulatory and cost of goods requirements. PfSPZ Vaccine comprises radiation attenuated, aseptic, purified, cryopreserved PfSPZ. In the 1st clinical trial in 80 volunteers it was safe, and well tolerated. However, it was sub-optimally immunogenic and protective due to inefficient administration. A 2nd product, PfSPZ Challenge, comprises non-irradiated, fully infectious PfSPZ. PfSPZ Challenge infected volunteers after intradermal administration by needle and syringe. However, administration was not optimally efficient. A 3rd product, PfSPZ-CVac, comprises PfSPZ Challenge administered to volunteers while receiving chloroquine chemoprophylaxis. Assessment of these three products in interactive and adaptive clinical trials will facilitate progress toward optimizing administration and dosage regimen of all three whole PfSPZ products, as well as those developed in the future from genetically altered parasites, thereby speeding licensure of one or more PfSPZ-based vaccines. In 2011-

2012 we will execute clinical trials of all three PfSPZ products at multiple clinical trials centers in N. America, Europe, and Africa. Plans and progress will be described.

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EVALUATION OF CHOLERA SEVERITY IN A HIGHLY AFFECTED HAITIAN POPULATION

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Exposure to toxigenic *Vibrio cholerae* O1 results in a wide spectrum of illness, from asymptomatic infection to severe disease. The variant strain of *V. cholerae* causing the epidemic in Haiti may be more virulent than previous El Tor biotype strains, which were estimated to cause severe disease in 2% of those infected. Grand Saline, a commune in Artibonite Department reported a high cholera attack rate (19.0%) between Oct. 16, 2010 and Feb. 19, 2011. We conducted a cross-sectional survey in this commune (estimated population 21,131) from Mar. 22 to Apr. 6, 2011 to characterize disease severity. We interviewed 2,543 residents ≥2 years old in 1,228 households selected by multistage sampling from 13 villages and collected serum from 2,464 (97%). The median age of participants was 23.5 years (range 2-90); 59.0% were female. A healthcare provider diagnosis of cholera was reported by 466 (18%) of all respondents, including 187 (18%) males and 279 (19%) females, and 239 (16%) of those <age 30 compared with 227 (21%) of those ≥30 years old ($p<0.01$). Any antacid use was reported by 103 (23%) of those with cholera versus 292 (15%) of those without cholera ($p<0.01$). Among the 466 respondents with a cholera diagnosis, 429 (92%) reported rice-water stool (median maximum 24-hour stool frequency = 7), 361 (78%) painful leg cramps, and 225 (48%) vomiting more than once. For treatment, 405 (87%) reported using oral rehydration salts, 315 (68%) receiving antibiotics, 213 (46%) receiving IV fluids, and 191 (41%) overnight hospitalization; 157 (33%) reported both IV fluids and hospitalization. Persons with severe disease (IV fluids and hospitalization) represented 6% of the study population. From a subset of 589 households with information about all 2,396 household members, 311 people were diagnosed with cholera (13.0%) and 13 cholera deaths were reported (case fatality ratio, 4%). Severe disease was common, occurring in 6% of the study population rather than 2% of those infected, as seen with previous El Tor strains. Serologic measures of exposure to *V. cholerae* will be examined.

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REVIEW OF CHOLERA CASES AND DEVELOPMENT OF QUALITY ASSURANCE TOOL FOR CHOLERA CARE AT HÔPITAL ALBERT SCHWEITZER, DESCHAPELLES HAITI

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The first cholera outbreak in Haiti in decades was confirmed this past October. Hôpital Albert Schweitzer (HAS) received a high volume of patients in the initial months of the epidemic, and records of patients meeting the WHO definition of cholera from October 20, 2010 to January 12, 2011 were reviewed. Demographic data included: age, sex, residence, and travel time to HAS. Clinical data included: days ill before presentation, length of stay, treatment protocol level (based on severity of condition at presentation), liters of intravenous fluid (IVF) received, use of antibiotics, concurrent illness, and outcome. Length of stay, use of antibiotics, liters of IVF, and protocol number were used as surrogate markers for illness severity. A total of 514 charts were located and reviewed. Mean age was 37 years, and 54% were male. The rural sections of Belanger, Liancourt, Terre Nette, and Bastien had the highest percentage of cases represented, with travel time to HAS of 10 minutes to 8 hours. Mean duration of illness before presentation was 1 day (range 0-6), and average length of stay was 3 days (range 0-15). A total of 78% of patients received IVF, with an average amount of 8.5L (range 0-47L). About half (47%) of patients received antibiotics, and overall mortality was 0.6%. Having a concurrent illness was a statistically significant predictor of all four markers of illness severity. Age was found to be a predictor of protocol assignment and antibiotic prescription. Based on information gleaned from this review and the comments of clinicians providing care, a quality assurance and documentation tool was developed for patient care. This tool consisted of a simple, single-page form, which included both documentation of the patient's clinical criteria at admission and a guide to assignation of treatment level. It is hoped that this review will contribute to better understanding of the ongoing epidemic and that utilization of a standardized form will increase efficiency of both patient care and data collection at HAS.

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PROTECTION AGAINST EPIDEMIC CHOLERA IN POST-EARTHQUAKE PORT-AU-PRINCE, HAITI, 2010

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On January 12, 2010, a magnitude 7.0 earthquake struck metropolitan Port-au-Prince, Haiti, destroying vital water and sanitation infrastructure and leaving 1.3 million people displaced. On October 21, 2010 toxigenic *Vibrio cholerae* O1 was confirmed to be the cause of a large outbreak of acute watery diarrhea in Artibonite department. On November 7, 2010, cholera was first reported in Port-au-Prince and by December 15, nearly 20,000 cases were reported in the city. We conducted a case-control study to examine exposures associated with cholera in this crowded urban environment. Between December 15-19, 2010, we enrolled cases who were persons ≥5 years old with acute, watery diarrhea admitted to the GHESKIO Cholera Treatment Center in the slum of Cité de Dieu and two age-, sex- and neighborhood-matched controls per case. We used a standard questionnaire to gather information about food and beverage exposures in the 3 days before illness onset, and water, sanitation and hygiene practices. We enrolled 53 cases and 106 controls. The median age of cases was 29 years (range 6-80); 45% were female. Controls were more likely than case-patients to have treated their drinking water by boiling or using a chlorine product before the outbreak began in Port-au-Prince (matched odds ratio [mOR]=0.3; 95% confidence interval [CI] 0.1, 0.9), and to demonstrate proper handwashing technique (lathering hands with soap and drying with a clean cloth or air [mOR=0.2; 95% CI 0.03, 0.7]). Food exposures that were implicated as risk factors for transmission in previous cholera outbreaks were not associated with illness in this investigation. Our investigation demonstrated that personal hygiene measures taken by individuals and families, including treating drinking water and proper handwashing, helped protect against disease in this urban cholera outbreak. Improvements in access to water and sanitation infrastructure should be a high priority for government and aid organizations in post-earthquake Port-au-Prince to protect against cholera and other diarrheal disease outbreaks.

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MEMORY B CELL RESPONSES TO LPS ARE ASSOCIATED WITH PROTECTION AGAINST INFECTION IN HOUSEHOLD CONTACTS EXPOSED TO *VIBRIO CHOLERAE* O1

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Vibrio cholerae is a noninvasive enteric pathogen responsible for rapidly dehydrating diarrhea. Further understanding of the immune mechanisms mediating protection may be necessary for the development of a vaccine that confers protection comparable to natural infection. We have previously shown that memory B cells develop after cholera, and have hypothesized that these cells may play a role in long-term protective immunity. To test this hypothesis, we examined whether the presence in the circulation of memory B cells in individuals exposed to *V. cholerae* O1 in a household with a cholera patient was associated with protection from subsequent infection in contacts of the index case. We analyzed memory B cell responses to both the protein antigen cholera toxin B subunit (CTB) and the non-protein antigen lipopolysaccharide (LPS) in a cohort of 236 household contacts of 122 index cases. We also analyzed baseline vibriocidal and plasma antibody responses against CTB and LPS for correlation with protection. As previously described, we found that higher baseline vibriocidal antibody levels correlated with a decreased risk of subsequent infection in contacts ($P \leq 0.001$). The presence of LPS-specific IgG memory B cells on exposure conferred a 68% decrease in the subsequent risk of infection in household contacts ($P = 0.032$). No protection was provided by the presence of IgG or IgA memory B cells to CTB or IgA memory B cells to LPS. Previous studies have shown that LPS-specific IgG memory B cells decline to baseline levels within one year following cholera, although protection against subsequent infection persists for several years. It is possible that memory to LPS at the mucosal surface may last longer than in the circulation and may mediate protective immunity.

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HIGH-THROUGHPUT PROTEOMIC-BASED SCREENING OF ANTI- *VIBRIO CHOLERAE* ANTIBODY RESPONSES IN HUMANS

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Vibrio cholerae causes an estimated 3-5 million cases and 100,000 deaths, annually. Although current vaccines have been shown to be safe and immunogenic, none provide the long-lasting protective immune responses seen with natural infection. Characterization of immunogenic *V. cholerae* antigens could lead to a better understanding of protective immunity in human cholera infection. Using a high-throughput proteomic-based platform called the Nucleic Acid Programmable Protein Array (NAPPA), we screened 3,761 *V. cholerae* open reading frames (97% of the ORFeome) for anti-*V. cholerae* IgG responses in 25 cholera patients,10 vaccinees who received whole cell-killed vaccine with recombinant cholera toxin (WC-rBS), and 10 North American volunteers. In our primary screen of cholera patients, we detected significantly higher IgG reactivity in convalescent sera to over 300 proteins including a number of previously identified immunogenic and virulence-associated proteins (e.g. cholera toxin B, CtxB; toxin co-regulated pilus A, TcpA; *V. cholerae* cytolysin, VCC/hlyA) when compared to acute sera, healthy Bangladeshis (pre-vaccine) and/or North American volunteers. We also identified several proteins which annotate as methyl-accepting chemotaxis (e.g. VC1069, VC0514), flagellin proteins FlaB and FlaC, heat shock protein HtpX, and several hypothetical proteins. A subset of proteins had significantly increased IgG responses at convalescence from infection, but no increased IgG immunoreactivity after vaccination. This list included OmpW, FlaB, FlgJ, and EpsL, EpsG and EpsE. Outer membrane protein W has been shown to be immunogenic and antisera in rabbits have been to shown to be protective in a rabbit ileal loop model. FlaB and FlgJ encode a flagellin and flagellar protein, respectively. EpsL, EpsG, and EpsE encode general secretion pathway proteins which are involved with secretion of cholera toxin and other virulence factors. These findings give insight into differences in immune responses elicited after natural infection and vaccination, and may aid in the development of improved cholera vaccination approaches.

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THE CASE FOR REACTIVE MASS ORAL CHOLERA VACCINATIONS, A DUKORAL AND SHANCHOL MODEL

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The massive outbreak of cholera in Haiti intensified interest in the control and prevention of cholera. Momentum for the use of OCVs has been magnified by the licensing in India of a killed whole-cell (WC) oral cholera vaccine without the B-subunit (Shanchol). This vaccine is available at low cost, administration does not require a buffer solution, immunity is acquired one week after the first dose, and the protective efficacy (PE) last for at least 36 months. Datasets of cholera outbreaks from three sites with varying cholera endemicity Zimbabwe, Kolkata (India), and Zanzibar (Tanzania) were analysed to estimate the number of cholera cases preventable with reactive vaccination under differing response times, vaccine coverage, and vaccine doses. The PE assumptions for Shanchol were 67% for 36 months, starting 1 week after completion of the first dose. Assumptions for the recombinant B-subunit containing WC vaccine (Dukoral) were 85% PE for the first 6 months, 60% up to 18 months and 20% up to the 36 month, starting one week after completion of the second dose. During a large cholera outbreak in Zimbabwe in 2008/9, 98,591 cholera cases were reported with 4,288 deaths. If a rapid response had taken place and half the population were vaccinated as many as 33,122 (34%) cholera cases and 1,391 (32%) would have been prevented with Shanchol and as many as 34,900 (40%) cholera cases and 1,695 deaths (40%) with Dukoral. With a delayed response Shanchol would have prevented more cases than Dukoral. In the sites with endemic cholera, Kolkata and Zanzibar, a significant number of cases could have been prevented with either vaccine but the impact less dramatic. Shanchol would have prevented more cases if the outbreak was extended. If the major peak of the outbreak occurred immediately following vaccination Dukoral would have prevented a larger proportion of cases. Once a substantial proportion of a population is vaccinated outbreaks in subsequent years may be reduced if not prevented. We show that reactive mass vaccinations are a rational response to cholera outbreaks in endemic and non-endemic settings using either Shanchol or Dukoral. Decision makers in donor and recipient countries should be made aware of the potential benefit of reactive cholera vaccinations.

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A COMPARISON OF MEMORY B CELL, ANTIBODY SECRETING CELL, AND PLASMA ANTIBODY RESPONSES IN YOUNG CHILDREN, OLDER CHILDREN, AND ADULTS WITH INFECTION CAUSED BY *VIBRIO CHOLERAE* O1 EL TOR OGAWA IN BANGLADESH

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Children bear a large component of the global burden of cholera. Despite this, little is known about immune responses to cholera in children, especially those under five years of age. Cholera vaccine studies have demonstrated lower long-term protective efficacy in young children. Memory B cell (MBC) responses may correlate with duration of protection following infection and vaccination. Here we report a comparison of immune responses in young children (3-5 years of age; n=17), older children (6-17 years of age; n=17) and adults (18-60 years of age; n=68) hospitalized with cholera in Dhaka, Bangladesh. We found that while young children had lower baseline vibriocidal antibody titers than adults (P=0.02), they had higher fold-increases between day 2 and day 7 (P=0.04). Young children had higher baseline IgG plasma antibody levels to *Vibrio cholerae* antigens (P=0.03 for cholera toxin B, P=0.05 for lipopolysaccharide), although the magnitude of responses at day 7 and 30 were similar across age groups. As a surrogate marker for mucosal immune responses, we assessed day 7 antibody secreting cell (ASC) responses. These were comparable across age groups, although there was a trend for older age groups to have higher levels of lipopolysaccharide specific IgA ASC responses (P=0.07). All age groups developed comparable MBC responses at day 30 to *V. cholerae* lipopolysaccharide and cholera toxin B subunit. These findings suggest that despite some differences, young children are able to mount robust vibriocidal, plasma antibody, ASC, and MBC responses against *V. cholerae* O1, suggesting that under an optimal vaccination strategy, young children could achieve protective efficacy comparable to that induced in adults.

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UNIQUE APPROACH TO THE MANAGEMENT OF DELUSIONAL PARASITOSIS

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Delusional parasitosis (DP) is a fixed, false belief that one is infested with parasites, worms, bacteria, mites, or other living organisms. DP may be a manifestation of underlying medical or psychiatric disorders (secondary DP), or can be a primary delusional disorder. DP can be effectively treated by neuroleptic medications, but patients with DP will generally refuse psychiatric referral. We describe our systematic yet simple approach to treating patients with DP at the Tropical Disease Unit (TDU) at Toronto General Hospital and report our results. Our first visit consists of a thorough medical assessment of the patient, as well as complete laboratory investigations to rule out both true parasitic infection and treatable causes of secondary DP. At follow-up ≥ 4 weeks later, if all investigations are normal, we introduce the idea that symptoms are due to a "chemical imbalance" that may have resulted from a previous parasitic infection, and suggest the use of neuroleptic agents to "rebalance" the patient's chemistry. The records of 82 DP patients who were referred to and assessed at the TDU between 1/2005 and 12/2010 and followed for at least 4 months were assessed retrospectively. There were 33 (40%) males and 49 (60%) females, with a combined mean age at clinical presentation of 54.0 years (SD 11.6, range 24-85). Our approach led to 75 patients (91.5%) agreeing to try a neuroleptic. Of these patients, 38 (50.7%) showed some response: 11 (14.7%) had a complete response with full resolution of delusions, 19 (25.3%) had a major response, and 8 (10.6%) had a minor response; 14 (18.7%) did not respond. Seventeen (22.7%) were lost to follow up, and 6 (8.0%) were ultimately treated by another physician. The majority of patients who responded to therapy (32/38, 84%) were treated with risperidone. Patient acceptance of our "chemical imbalance" approach has been highly successful in our centre, although the effectiveness of therapy remains suboptimal.

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NEWLY RECOGNIZED CLINICAL SYNDROMES OF SNAKE BITE ENVENOMING

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Snake bites can no longer be dismissed as being too rare and inconsequential to deserve the time and attention of practitioners of tropical medicine. Recently published national epidemiological surveys estimated about 50,000 snake bite deaths each year (0.5% of all deaths) in India and 6,000 in Bangladesh. Even when geographically-appropriate polyspecific antivenom is available, species identification is crucial to allow anticipation, early treatment and prevention of life-threatening complications. Recognition of a characteristic clinical syndrome contributes to diagnosis when examination of the snake and rapid immunodiagnosis are impossible. However, the range of clinical features of envenoming associated with each taxon is broadening. Multi-focal thrombotic microangiopathy, causing cerebral and other infarctions, has been documented in victims of the two Lesser Antillean lanceheads, Asian Russell's vipers, some North American rattlesnakes, African puff adders and lowland vipers. In South Asian countries, kraits menace those sleeping on the floors of their homes. In the Indian sub-continent, severe abdominal pain is the dominant presenting symptom of envenoming by common kraits but its mechanism remains unknown. In Bangladesh, rhabdomyolysis leading to fatal acute kidney injury (AKI) was caused by bites of greater black kraits, a species previously unknown in that country. In Vietnam, patients envenomed by two species of krait developed fatal hyponatremia as well as hypertension, rhabdomyolysis and persistent mydriasis. Envenoming by some species of coral snakes in North and South America may produce unexpected effects including rhabdomyolysis, coagulopathy, gastro-intestinal and urinary tract bleeding, hemolysis and excruciating local pain. Atypical clinical presentations, without the expected paralysis, have been observed recently in victims of two classically neurotoxic species: severe local envenoming and coagulopathy caused by Eastern green mambas and rhabdomyolysis and AKI caused by smooth-scaled death adders in New Guinea. A hemolytic-uremic-syndrome-like picture of microangiopathic hemolysis with thrombocytopenia and AKI has been described with envenoming by some African and Indian Viperidae and Australian Elapidae. These findings demand radical revision of currently accepted concepts of the symptomatology associated with envenoming by various species of snakes.

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URINARY TRACT INFECTIONS AMONG PREGNANT WOMEN IN COAST PROVINCE, KENYA

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Urinary tract infection (UTI) is common in pregnancy, affecting 6-16% of women worldwide. Maternal pyelonephritis, low birth weight, and preterm birth are associated with UTI in pregnancy, so prompt diagnosis and treatment is essential. In much of the developing world, the rates of UTI and offending organisms are poorly described, as urine culture is not routinely performed. Our aim was to understand the profile of UTI during pregnancy in the region to inform diagnosis and treatment decisions. 159 expectant mothers were recruited at their first antenatal visit from March-May 2011 from the antenatal clinic at Msambweni District Hospital in Coast Province, Kenya. Mothers were asked about history of UTI and current UTI symptoms, and midstream clean-catch urine was collected. Urine was analyzed by dipstick, microscopy, and urine culture. Antibiotic sensitivities were determined by Kirby-Bauer method. Of 159 women, 29 (18.2%) had significant bacteriuria (>10^5 CFU/ml), and another 18 (11.3%) had a lesser degree of bacteriuria (>10^4 CFU/ml). Thirty-eight percent (38%) of recruited women had UTI symptoms, and 17% had a history of UTI. The most frequently isolated bacteria were *S. aureus* (46% of positive cultures), *Enterococcus* (21%), *E. coli* (16%), and *Klebsiella* spp. (12%), which demonstrated 4%, 40%, 38%, and 33% resistance, respectively, to ampicillin, the most commonly prescribed antibiotic. Other pathogens detected on microscopy included *Schistosoma haematobium* (6.4% of women), *T. vaginalis* (3.6%), and *Candida* spp. (6.4%). A combination of dipstick (nitrite and leukocyte esterase (at least 2+)) and microscopy (>5 leukocytes/hpf) was 75% sensitive and 43% specific for detecting bacterial and non-bacterial UTIs. Our findings suggest that levels of UTI among pregnant women in our study population are somewhat higher than rates recorded in other developing countries, and *S. aureus* bacteriuria comprises a higher percentage of infections than previously reported in the literature. The current screening method (dipstick + microscopy) is relatively sensitive for detection of UTIs, but it lacks specificity, leading to unnecessary antibiotic usage and possibly increased antibiotic resistance. Urine culture with moderately priced media (i.e. CLED agar) should be considered to confirm positive UTI screens, minimizing the sequelae of UTI in pregnancy and the development of bacterial antibiotic resistance.

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A NOVEL BUBBLE CPAP DEVICE FOR LOW RESOURCE SETTINGS

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Respiratory distress and failure are leading causes of infant morbidity and mortality in the developing world. Infants who are in respiratory distress can be supported with methods of non-invasive ventilation such as bubble Continuous Positive Airway Pressure (bCPAP); however, bCPAP devices are not readily available in low resource settings due to high cost -- on average, $6,000 -- and technical complexity. To address this need, we engineered a novel bCPAP system which can be made at a unit cost of only $160. Moreover, because of its simple design, it requires only the replacement of a $1 diaphragm approximately every 2 years to maintain. The novel bCPAP device consists of an adjustable flow generator and a pressure-regulated delivery system. The adjustable flow generator, consisting of two diaphragm pumps, provides a continuous flow of ambient air that is controlled with a standard flow regulator. If supplemental oxygen is required, the output of an oxygen concentrator or tank can be connected to an input port in the flow generator. By adjusting the flow of ambient air and oxygen, the % oxygen administered can be controlled. The pressure-regulated delivery system, consisting of nasal prongs and a pressure control tube submerged in a column of water, controls the air pressure that is delivered. The objective of this study was to evaluate the output pressure of this novel bCPAP device. The nasal prong pressures of the novel bCPAP device and a comparable, bCPAP system at the Texas Children's Hospital (TCH) were measured using a digital pressure sensor and collected continuously over a 2-minute period. At a flow setting of 7 L/min and a water column level of 6cm, nasal prong pressure data of the novel bCPAP device and of the TCH bCPAP system indicate equivalent output pressure ranges. The novel bCPAP device provides a method for low-cost, easy-to-use and repair respiratory support and has the potential to successfully treat respiratory distress in infants and small children in low resource settings.

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TRAVEL-RELATED ILLNESSES AMONG PEDIATRIC TRAVELERS WHO VISIT FRIENDS AND RELATIVES (VFRS) IN CANADA

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Travelers who visit friends and relatives (VFRs) are at increased risk of travel-related illnesses (TRIs); however, there is little data regarding travel-related illnesses among pediatric VFRs. The Canadian Paediatric Surveillance Program (CPSP) is an active national surveillance program that collects data from approximately 2,500 pediatricians and pediatric sub-specialists in Canada. We undertook a two year surveillance project through the CPSP of all cases of significant travel-related illness among pediatric VFRs in Canada from March 2009 to February 2011. Mild respiratory and gastrointestinal illnesses were excluded. There were 88 confirmed cases of significant travel-related illnesses among pediatric VFRs in Canada. Among the pediatric TRIs, 64% were acquired in Asia, 21% in Africa, 12% in Central/South America and the Caribbean, 2% in the Middle East and 1% in Europe. The average duration of travel was 7 ½ weeks. Enteric fever (31 confirmed and 5 presumed cases) was the most common TRI. Malaria (17 cases) and hepatitis A (11 cases) were the next most common TRIs. Fever was the most common presenting symptom in 80% of children with TRIs. Three patients presented with hypotension (malaria) or septic shock (Salmonella bacteremia). There was one death and four children had significant sequelae following their TRIs. Three quarters of cases required hospitalization (n=67) with an average length of stay of 11 days (median 5.5 days). The majority of pediatric VFR travelers did not seek pre-travel advice (73%) and among those who did, only 1/3 sought advice from a travel health clinic. Our data indicate that TRIs cause significant morbidity and some mortality among pediatric VFRs in Canada. Furthermore, the majority of the TRIs were potentially preventable if appropriate pre-travel advice had been obtained and followed. This highlights the need for increased education of families and health care providers regarding the importance of pre-travel advice to minimize the risk of acquiring travel-related illnesses among pediatric VFRs.

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PREDICTING ADVERSE OUTCOMES AMONG PEDIATRIC INPATIENTS IN AN AREA OF LOW MALARIA TRANSMISSION IN TANZANIA

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The proportion of infants and children living in areas of low malaria transmission intensity in sub-Saharan Africa is increasing. To improve child health outcomes, methods for identifying severe illness need to be evaluated in areas where malaria is uncommon. We identified febrile pediatric patients among consecutive admissions in Moshi, Tanzania from September 2007 to August 2008, recorded clinical data using Integrated Management of Childhood Illness (IMCI) criteria, and collected diagnostic specimens. Of 466 participants with known hospital outcomes, median age was 1.4 years (range 2 months-13 years), 266 (57.0%) were male, 11 (2.4%) had malaria, and 34 (7.3%) died. Inpatient death was associated with Blantyre coma score <5 (OR 23.2, p<0.001); central cyanosis (OR 20.6, p=0.003); capillary refill >3 seconds (OR 9.0, p<0.001); inability to breastfeed/drink (OR 8.9, p<0.001); stiff neck (OR 7.0, p<0.001); bulging fontanelle (OR 6.5, p=0.024); severe anemia (OR 5.3, p<0.001); lethargy (OR 5.2, p<0.001); severe wasting (OR 4.9, p <0.001); skin pinch >2 seconds (OR 4.8, p=0.003); abnormal breath sounds (OR 4.3, p<0.001); signs of respiratory difficulty (OR 4.0, p<0.001); history of fever >7 days (OR 3.8, p<0.001); generalized lymphadenopathy (OR 3.6, p=0.005); oral candidiasis (OR 3.4, p=0.015); history of convulsions in past 48 hours (OR 2.8, p=0.017); referral from another inpatient facility (OR 2.5, p=0.010); low mid-upper arm circumference aged ≥6 to <60 months (p=0.003); and low weight-for-age Z score (p=0.014). Factors not associated with death included: hypoglycemia (OR 6.4, p=0.206); hepatomegaly (OR 2.5, p=0.068); severe pallor (OR 1.8, p=0.288); splenomegaly (OR 1.5, p=0.517); jaundice (OR undefined, p=1.00); and positive malaria film (OR undefined, p=1.00). Overall IMCI criteria performed well for predicting in-hospital death although typical malarial signs had little value. Health care workers should be trained to recognize differences in the constellations of clinical signs associated with severe illness in an area of low malaria transmission.

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THE CONTRIBUTION OF MALARIA RETINOPATHY TO REDUCING MORTALITY FROM *PLASMODIUM FALCIPARUM* MALARIA IN ASIA

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The treated mortality of severe malaria remains high. Adjunctive therapies aiming to reduce this should target pivotal aspects of its pathogenesis. However, understanding of the pathogenesis is incomplete and there is a lack of surrogate markers for use as endpoints in treatment trials. Management is hampered by difficulty distinguishing malarial from non-malarial coma and predicting prognosis. Detailed description of malaria retinopathy can address many of these issues. We describe a series of observational studies of malaria retinopathy in Bangladesh and India in 2008-2011. The aims were to assess the potential contribution of retinopathy to diagnosis of malarial coma, assessing prognosis, understanding pathogenesis and as a surrogate marker for adjunctive treatment studies. Admitted smear positive patients with *P. falciparum* malaria of any severity plus control groups of febrile encephalopathy, sepsis and healthy volunteers were recruited. Patients' retinas were photographed daily until discharge then weekly until normal. Detailed clinical assessment, markers of and contributors to microcirculatory obstruction (plasma lactate, rectal capillary blood flow, PfHRP2 and red blood cell stiffness), fluorescein angiography and cerebral MRI were performed. 287 patients were enrolled (192 with malaria, and 30 in each control group). Retinopathy was most common and severe in cerebral (85%) and fatal (90%) malaria and correlated with severity of malaria. It was specific for cerebral malaria in comatose patients (94%), and its severity correlated with coma recovery time. It resolved in 2 weeks but visual function recovered in 3-4 days. Malaria retinopathy correlated with underperfusion and blood retinal barrier (BRB) leakage on angiography; plasma lactate, red cell stiffness and PfHRP2-derived parasite biomass. Malaria retinopathy is a bedside tool which can help diagnosis and prognosis in patients with cerebral malaria. Retinopathy is caused by microvascular obstruction and BRB leakage and by serial assessment can be used as a surrogate marker for intervention studies.

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ENDOCRINE PANCREAS AND *TRYPANOSOMA CRUZI* - A LINK TO DIABETES?

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Trypanosoma cruzi infection (Chagas disease) affects millions of people in South and Central America. Recently, Chagas disease has become a public health concern in non-endemic areas including the United States due to immigration. So far researches have been focused on the studies on *T. cruzi*-induced cardiomyopathy and digestive isorders. Here in for the first time we have shown that *T. cruzi* infection may have a direct link to insulin secretion and metabolic disorders. CD1 mice infected with Brazil strain trypomastigotes had a significant decrease in insulin levels (3.5 fold) compared with uninfected mice. Reduced levels of insulin persist into the chronic stage (day 133 post infection). We measured Insulin response to the administration of L-arginine in infected and infected mice. L-arginine stimulated acute phase of insulin secretion in uninfected mice

but the response in infected mice was significantly reduced in acute and chronically infected mice. Administration of the beta-3 adrenergic receptor agonist (CL316,243) increased plasma insulin levels (4 fold) in uninfected mice but the response in infected mice was reduced. This was observed in acute and chronically infected mice. Histopathological studies displayed the presence of parasites in the acinar cells of pancreas during acute infection. H&E staining of the pancreas revealed a massive inflammation during acute infection which persisted into the chronic phase. The pancreas synthesizes several hormones including insulin, glucagons and somatostatin thereby governing carbohydrate and lipid metabolism. Malfunctioning of pancreas during *T. cruzi* infection may cause insulin resistance and diabetes.

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IFN- Γ REDUCES CELLULAR IRON INTAKE THROUGH REGULATION OF IRP2 EXPRESSION, A WAY TO CONTROL *LEISHMANIA INFANTUM CHAGASI* REPLICATION IN MACROPHAGES

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Visceral leishmaniasis (VL) is endemic in Brazil and *Leishmania infantum chagasi* is the main etiological agent. Dogs are the main domestic reservoir of leishmania and, like humans, develop a spectrum of clinical forms that varies from asymptomatic to severe and fatal disease. When there is intracellular iron depletion, iron regulatory protein 2 (IRP2) binds to transferrin receptor mRNA, allowing its translation, which results in increased iron intake by the cell. *L. i. chagasi* infected cells tend to be depleted of iron. Our hypothesis is that iron scavenging is impaired by IFN-γ which limits *Leishmania* intracellular proliferation. Blood and spleen fragments were collected from euthanized dogs with VL (n=31). qPCR was used to assess IRP2, IFN-γ and IL-10 gene expression in spleen RNA. Parasite load was evaluated in spleen by qPCR for kDNA. ANOVA test followed by Tukey post-test was used to assess differences among the groups and Spearman test was performed with a significance of 5%. Dogs were grouped by parasite load: Group 1 (n=6), <1,000 parasites/spleen µg; Group 2 (n=7)1,000-10,000; Group 3 (n=6) 10,000-100,000 and group 4 (n=12) >100,000. Analysis was performed comparing group 1 with the other groups. This study was approved by Ethical Committee for Animal Research of Federal University of Rio Grande do Norte. IRP2 expression in groups 2 and 3 was increased two (p=0.0162) and four fold (p=0.0066), respectively, whereas IFN-γ decreased 4 and 5 fold in the same groups (p=0.0385; p=0.0195). An inverse correlation between IFN-γ and IRP-2 was observed (r=-0.906). Conversely, IL-10 was directly correlated with the parasite load (r=0.577), in groups 2 (p=0.0095) and 3 (p=0.0071). Spleen cells from dogs with lower leishmania load presented higher expression of IFN-γ and decreased expression of IRP2. Thus, IFN-γ-induced iron depletion in the macrophage may be a mechanism for control *L.i.chagasi* replication by host cells.

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B7-1/B7-2:CD28/CTLA-4 COSTIMULATION PLAYS A CRITICAL ROLE IN ESTABLISHMENT OF CHRONIC *LEISHMANIA MEXICANA* INFECTION IN C57BL6 MICE

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Using mice deficient in B7-1, B7-2 or both molecules, we had previously shown that B7-2 mediated pathway induces IL-4 production and plays a role in pathogenesis of *L. major* infection in BALB/c mice. However, the role of B7-1/B7-2:CD28/CTLA4 co-stimulation pathway in infections caused by other *Leishmania* species such as *L. mexicana* is not clear. In this study, we analyzed the role of B7/CD28 family molecules in *L. mexicana* infection by monitoring cutaneous growth of *L. mexicana* in B7-1$^{-/-}$/B7-2$^{-/-}$, CD28$^{-/-}$/CTLA4$^{-/-}$, PDL1$^{-/-}$, PDL1$^{-/-}$/CD28$^{-/-}$/CTLA4$^{-/-}$ and wild type (WT) C57BL6 mice. Following s.c. inoculation with *L mexicana* amastigotes into back rump, B7-1$^{-/-}$/B7-2$^{-/-}$ mice developed no lesions or significantly smaller lesions containing significantly fewer parasites compared to similarly infected WT mice. CD28$^{-/-}$/CTLA4$^{-/-}$ mice developed lesions similar to WT mice during early course of infection but resolved them eventually. Interestingly, CD28$^{-/-}$/CTLA4$^{-/-}$ mice lacking PDL1 (PDL1$^{-/-}$/CD28$^{-/-}$/CTLA4$^{-/-}$) were highly susceptible to *L. mexicana* infection similar to WT mice and PDL1$^{-/-}$ mice. Unlike the WT mice which had high serum titers of parasite specific IgG1 both the B7-1$^{-/-}$/B7-2$^{-/-}$ and CD28$^{-/-}$/CTLA4$^{-/-}$ C57BL6 mice did not elicit significant antibody titers during the course of infection. At week 13 post-infection, *L. mexicana* antigen-stimulated lymph node cells from CD28$^{-/-}$/CTLA4$^{-/-}$ mice produced more Th1 associated IL-12 and IFN-γ compared to similarly stimulated lymph node cells from WT mice which produced significantly higher levels of Th2-associated IL-4. Lymph node cells from PDL1$^{-/-}$/CD28$^{-/-}$/CTLA4$^{-/-}$ mice did not elicit significant levels of IFN-γ, IL-12 nor IL-4 at this time point. Taken together, these results indicate that B7-1/B7-2:CD28/CTLA-4 interaction plays a critical role in pathogenesis of cutaneous leishmaniasis caused by *L. mexicana* by inducing disease exacerbating Th2 response and IL-4 production. Furthermore, our findings suggest that PDL1 associated pathway contributes to enhanced resistance of CD28$^{-/-}$/CTLA4$^{-/-}$ mice against *L. mexicana*.

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SARCOCYSTIS SPECIES IN MALAYSIA: A MOLECULAR CHARACTERIZATION USING DNA PROFILLING

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This study was carried to establish the DNA profile of *Sarcocystis* species found in wild rodents in Peninsular Malaysia. One hundred and forty six rodents belonging to 7 species trapped in the states of Johor, Selangor, Kelantan and Kedah were examined. Rodents as an intermediate host to *Sarcocystis* pose a public health. Studies have shown the prevalence rate of *Sarcocystis* in Southeast Asia to be high. Human infections with Sarcocystis spp. from rodents results in human muscular sarcocystosis, implicated with myalgia, erythematous subcutaneous nodules, fever, bronchospasm, cough, headaches, loss of appetite, weight loss and lethargy. Hematoxylin and eosin (H&E) stained sections of the tissues from the wild rodents showed the presence of *Sarcocystis* species. In the study using light microscopy, a total of 146 thigh muscles were examined and 73 (50%) were found to be positive. Morphological observation showed that there may be 3 different species infecting these rodents. The brain sections were found not to contain any cysts. To identify the species present in these wild rodents, DNA extraction was carried out on paraffin embedded blocks of tissue using 5 prime archive pure DNA cell/tissue kit. DNA profiling was done for the identification of the different species. The results of the analysis will be reported at the

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IMPORTANCE OF UBIQUITIN AND UBIQUITIN LIKE PROTEIN MODIFIERS (UFM1) CONJUGATION IN THE PATHOGENESIS OF *LEISHMANIA DONOVANI*

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Leishmaniasis is a spectrum of diseases caused by protozoan parasites belonging to several different *Leishmania* species. There are no effective vaccines against leishmaniases. Currently available therapeutic regimens are often limited in effectiveness due to unwarranted side effects and

rapidly emerging drug resistance. Therefore, the quest for a novel vaccine and therapeutic targets acquires urgency towards controlling leishmaniases. Ubiquitin and ubiquitin like protein modifiers (Ubls) regulate a variety of biological functions ranging from endocytosis, membrane trafficking, protein kinase activation, DNA repair and chromatin dynamics. Studies of Ubl functions in human parasitic organisms are limited. Recently, we described the existence of a novel Ubl named ubiquitin-fold modifier 1 (Ufm1) that conjugates to parasite proteins in *Leishmania donovani*. Our studies showed that the Ufm1 conjugation system is mitochondria associated and conjugation of Ufm1 to mitochondrial proteins occurs in a parasite stage-specific manner. We also showed that modification/alteration of proteins that regulate Ufm1 conjugation reaction results in reduced survival of *L. donovani* in infected human macrophages suggesting their role in *Leishmania* pathogenesis. In order to further elucidate the biological roles of the Ufm1-modification, we prepared an Ufm1 null mutant (ufm1-/-). Consistent with the earlier results, the ufm1-/-mutants showed reduced survival in amastigote stage and this defect was reversed by re-expression of wild type but not the non-conjugatable Ufm1 indicating the essential nature of Ufm1 conjugation reactions. Results including ultra-structural studies and the effects of absence of Ufm1 on the cell cycle regulation in *L. donovani* will be discussed. Further, ufm1-/- parasites also provide an opportunity to explore such parasites as live attenuated vaccine candidates.

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CHARACTERIZATION OF A NOVEL INVARIANT METACYCLIC SURFACE PROTEIN OF *TRYPANOSOMA BRUCEI BRUCEI*

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African trypanosomes of the *Trypanosoma brucei* complex undergo several differentiation steps during development in the tsetse fly, including differentiation to the procyclic form in the fly midgut, epimastigote forms in the proventriculus, and ultimately to mammalian infective metacyclic trypomastigotes in the salivary glands. Finally, after inoculation into the vertebrate host, metacyclic parasites develop into the bloodstream form. A recent in-silico screen of the *T. brucei* genome yielded 111 unknown proteins containing glycosylphosphatidylinisotol (GPI) anchor structures. GPI anchors typically bind proteins to cell membranes and as such, these hypothetical proteins may be expressed on the parasite cell surface. A semi-quantitative gene expression analysis performed on parasite infected salivary glands, proventriculus, and midguts, as well as bloodstream parasites looking for preferential expression in particular developmental stages was recently performed. This analysis revealed a trypanosome gene family specifically upregulated in infected salivary glands. Importantly, these genes were found to be expressed in the mammalian infective metacyclic form collected from tsetse saliva. This gene family was further characterization at the RNA and protein level in both tsetse fly, and mammalian host immediately after transmission. These data support the possibility of identifying novel transmission-blocking targets from investigations into tsetse salivary gland trypanosome stages. Importantly, these data shed light on the parasite biology immediately after transmission to the vertebrate host.

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IMMUNOLOGICAL DETERMINANT UNDERLYING THE CONTROL OF INFECTION IN HUMANS INFECTED BY *TRYPANOSOMA BRUCEI GAMBIENSE*

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Human African Trypanosomiasis or sleeping sickness is caused by *Trypanosoma brucei gambiense* and *T. b. rodhesiense* parasites that are transmitted to humans by tsetse flies. As for many infectious diseases it is now clear that a wide range of outcome may result from the infection by trypanosomes. The disease is classically characterised by an early haemolymphatic phase (stage 1) followed by a meningoencephalitic phase (stage 2) leading to neurological disorders and death if left untreated. However, in *T.b. gambiense* endemic area where mass screening of the population is routinely performed by the Card Agglutination Test for Trypanosomiasis (CATT), a high proportion of individuals displaying positive serological results are negative to direct parasitological investigations. Increasing evidence now indicate that at least part of these subjects are infected but harbour parasitaemia levels that are below the detection limit of the parasitological tests used in the field, suggesting that they are able to control infection. The nature of the immune response in these individuals has yet received poor attention. In this communication we report on the quantification of the cytokine levels (IL-12, IL-2, IL-4, IL-5, TNF-α, INF-γ, IL-8, IL-1β, IL-6, IL-10) measured in healthy endemic controls, stage 1 and stage 2 patients and on a cohort of seropositive subjects from Guinea that were followed up in time to assess the evolution of their parasitological status. Whereas HAT patients were characterized by elevated levels of IL-1β and IL-10, seropositive subjects exhibited high levels of IL-6, IL-8 and TNF-α and low levels of IL-1β, IL-12 and IL-10. Interestingly high levels of IL-10 in seropositive subjects were also associated with an increased risk of developing the disease in this category of subjects.

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A NOVEL, FULLY AUTOMATED SAMPLE-TO-RESULT REAL-TIME PCR ASSAY FOR POINT-OF-CARE DETECTION OF DENGUE VIREMIA

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Appropriate treatment of patients with dengue relies on early clinical recognition. Laboratory methods to confirm dengue virus (DENV) infection are time-consuming, labor-intensive, and require a high level of technical skill. RT-PCR, the most sensitive method for DENV detection, has been difficult to transfer to the clinical setting; the lack of integration and automation of nucleic acid tests has been one major obstacle. We have developed a fully-automated, rapid qualitative RT-PCR assay for the detection of dengue viremia based on IQuum's lab-in-a-tube (Liat™) platform, which integrates raw sample processing and detection, including target enrichment, inhibitor removal, nucleic acid extraction, reverse transcription and real-time PCR, in a single closed-tube format with a turnaround time of <35 minutes. We tested the performance of the assay using serum from known DENV-infected subjects collected as part of a

passive dengue surveillance program, as well as archived serum from healthy North American donors enrolled in two separate non-flavivirus vaccine studies. Serum vials were thawed and 150 µl per sample were extracted and run on the Liat platform. Assay results were compared to those using a published benchtop real-time RT-PCR method after RNA extraction. In comparison to the reference benchtop PCR assay, the Liat Assay detected DENV RNA in 46 of 46 PCR-positive samples, as well as in 8 of 10 samples with equivocal results (including 4 of 4 samples from IgM+ subjects). The Liat results were negative in all 6 samples where the reference assay yielded unequivocal negative results, and in all 31 samples from the North American study volunteers. More testing of archived and live samples is in progress, yet the preliminary testing of the Liat Dengue Assay suggests a high degree of sensitivity and specificity compared to traditional laboratory-based methods for detection of dengue viremia. When this is confirmed, the ease-of-use and fast speed of this Liat test will enable greater access to nucleic acid testing in decentralized settings.

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PERMISSIVENESS OF BONE MARROW PROGENITOR CELLS FOR DENGUE VIRUS INFECTION

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Dengue is one of the most important mosquito-borne viral diseases affecting humans, with over half of the world's population living in areas at risk. Bone marrow suppression associated with reduction of megakaryocytes has been observed in dengue patients during the acute stage of infection. Studies of bone marrow biopsies from patients during acute infection indicate dengue virus infection induces hypocellularity in bone marrow progenitor cells. *In vitro* investigations also revealed that bone marrow cells were highly permissive for dengue virus infection. Results from early attempts to investigate the possible underlying mechanisms leading to bone marrow suppression have been inconclusive. A systematic investigation on this subject was performed with bone marrow from 20 healthy rhesus monkeys. Freshly collected bone marrow aspirates were infected with low dose (MOI=0.1) of dengue virus, strain 16881 grown in Vero cells. Cell smears were performed and supernatant fluids collected daily for 10 consecutive days or on days 1, 2, 3, 5, 7, 10, and 14 after infection. NS1 concentration was quantified by ELISA and viral titers were measured by quantitative real-time RT-PCR. Supernatants obtained on days 2 and 5 were used to co-culture with Vero cells to evaluate the infectivity of the virus. Immunohistochemical staining with antibodies for cell surface markers and dengue viral antigen was performed on smears. Electron microscopy was used to evaluate viral particle in infected BM cells. Results revealed that bone marrow cells were i) highly permissive for dengue virus infection, especially from young monkeys; ii)the peak in viral titers were observed on day 3 after infection, which corresponded to the peak in NS1 concentration; and iii) infectious virus could be recovered predominantly from day 2 but less often from day 5. Surface marker staining of sequential daily samples indicated that progenitor cells expressing CD41 markers were positive for dengue viral antigen early (1-3 days) post infection, while cells with markers typical for dendritic cells or macrophages were positive for dengue viral antigen at later time periods (days 5-8) of infection. The significance of the findings will be discussed.

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DEVELOPMENT OF A RAPID, SPECIFIC AND SENSITIVE PCR-MICROSPHERE BEAD ASSAY FOR DIFFERENTIAL DETECTION OF DENGUE VIRUS SEROTYPES

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Differential diagnosis of dengue viruses (DENV) is typically conducted using the plaque reduction neutralization test (PRNT) or virus isolation followed by serotype-specific immunofluorescence assay, real-time RT-PCR, or sequencing. Although, PRNT, RT-PCR and sequencing are specific, they require several hours to days to obtain reliable results. Therefore, to provide rapid diagnosis of DENV infection, we developed a differential multiplex PCR-microsphere bead assay (PCR-MBA) assay to detect DENV-1, -2, -3, and -4 serotype-specific RNA. The PCR-MBA employs pan-flavivirus primers, a one step RT-PCR, serotype- and virus-specific probes, and the Luminex platform to differentiate between DENV-1 to -4 serotypes and five other related flaviviruses. The assay was validated for DENV using qRT-PCR, virus isolation, sequencing, and/or PRNT using 85 well-characterized serum specimens obtained from patients and healthy controls with wide age range and geographical distribution. The DENV PCR-MBA successfully identified 85 serum specimens (100%) with differential detection of DENV-1, -2, -3, and -4. Results for the remaining five arboviruses were in accordance with the serum panel and were negative with low background values. Based on analysis of 85 serum specimens, the specificity, sensitivity, accuracy and negative predictive value for the PCR-MBA was 100% when compared to in-house confirmatory tests; qRT-PCR, RT-PCR, virus isolation, PRNT, and sequencing. These data indicate that the DENV PCR-MBA is both sensitive and specific when tested using a panel of DENV-1, -2, -3 and -4 positive serum samples and a large panel of negative controls. The primary advantage of the PCR-MBA reported here is the rapid detection of DENV, multiplex detection of DENV serotypes circumventing immunofluorescence assay and PRNT for DENV serotyping, ability to concurrently test up to 96 samples, and use of the Luminex platform that provides a high degree of automation and standardization. This assay has potential to be employed for simultaneous detection of other related arboviruses.

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EVASION STRATEGIES AND EARLY DETECTION OF DENGUE VIRUS (DENV) BY PRIMARY HUMAN DENDRITIC CELLS

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Production of type I interferon (IFN), is an essential first engagement of the host innate immunity for the control of many viral infections. The induction of these fundamental cytokines is initiated upon detection of virus by the cellular pattern recognition receptors (PRRs). Dengue Virus (DENV), the most prevalent arbovirus in humans, can modulate the host immune response using both, passive escape strategies, such as hiding its replication products from the host PRRs, and also in an active fashion, expressing factors that antagonize the cellular innate immunity. The main mechanism of immune evasion by DENV, described by several groups, is the interference of the type I IFN signaling pathway. Recently our laboratory reported the inhibition of type I IFN production in human monocyte derived DCs (mDCs), with an otherwise strong cytokine and chemokine profile in those cells. On a subsequent report we demonstrated that the NS2B3 protease complex of DENV, functions as an antagonist of type I IFN production in mDCs, and its proteolytic activity is necessary for this function. We are currently performing target pull down experiments using the tandem affinity purification strategy with different versions of the protease complex (NS2B3) as a bait to further identify possible

cellular targets and characterize the mechanism of inhibition of Type I IFN production in human immune cells by this viral product. Additionally, we are analyzing the immune response in human DCs, using primary isolates of DENV and a series of deletion mutants of the 3' untranslated (UTR) region. Our preliminary published results show that specific deletions in this region, previously shown to affect replication in some mammalian cells may also confer a rearrangement of the viral RNA structures that could be sensed differently by the cellular PRRs, as described for other pathogens.

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DENGUE VIRUS TRANSMISSION VIA MICROPARTICLES SHED FROM INFECTED MEGAKARYOCYTIC AND ERYTHROCYTIC PROGENITOR CELLS

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Dengue Virus is one of the most important vector-borne human pathogens, causing the most mosquito-transmitted infections, leading to 50-100 million hospitalizations a year. Disease may manifest in many forms, ranging from asymptomatic to life-threatening illnesses, such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). One key characteristic of dengue disease in humans is the presence of viremia, which may last as long as seven days. The patient viral load, measured by qRT-PCR, may reach as high as log 9 genome equivalents per ml of whole blood. Despite this high level of infectious virus, there are virtually no detectable classical viral particles that can be visualized by electron microscopy (EM). Recent evidence from our lab has shown virus like particles inside of platelets and the presence of free vesicles concentrated from the serum of infected patients. This work suggests that the morphology of virus propagated in humans differs from the structures observed from cell culture systems, such as Vero. Our investigation has extended to *in vitro* dengue virus infections with the megakaryocytic and erythrocytic progenitor cell lines, K562 and Meg-01. EM results demonstrate that the morphology of virus in the supernatant from these cells are vesicle-like, consistent with that observed from human patient serum. Transmission EM analysis demonstrates the presence of dengue envelope positive membrane vesicles, or DV-MPs, from the supernatants of virus-infected cells. The DV-MPs from these cells are positive for dengue virus by fluorescent cell staining. Radio-labeling experiments show that serum from convalescent dengue patients can recognize a unique 55kDa protein from DV-MPs derived from infected K562 and Meg-01. Infectious virus can be recovered from the DV-MPs isolated from these cell lines as well as from dengue patients by co-culture with Vero cells. This data suggests that dengue virus may be disseminated *in vivo* through vesicle membranes shed from the surface of infected cells.

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BIOMARKERS PREDICT PROGRESSION OF UNCOMPLICATED DENGUE FEVER TO DENGUE HEMORRHAGIC FEVER IN BUCARAMANGA, COLOMBIA

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Dengue represents the most important arboviral infection worldwide and is of increasing global importance. The development of hemorrhagic manifestations in dengue fever is associated with increased mortality. Therefore, biomarkers that improve prediction of individuals in need of referral and hospitalization could lead to better clinical decision making and reduced mortality. This study was undertaken to determine if i) perturbations in host biomarkers from pathways of known pathogenesis in dengue fever can identify individuals at-risk of clinical deterioration; and ii) host biomarkers, when combined with useful clinical parameters, can improve clinical assessment of dengue patients early in disease. Using a case-control design, we randomly selected subjects from a prospective cohort study of acute dengue in Bucaramanga, Colombia. Using serum collected from subjects within the first 96 hours of illness, we tested 18 biomarkers by ELISA in cases (DHF, n=46; subjects with dengue fever who developed hemorrhagic manifestations) compared to controls (DF, n=66; subjects with uncomplicated dengue fever). sICAM-1, sEndoglin and IP-10 were elevated in subjects who developed DHF (p=0.009, p=0.022 and p=0.014 respectively). In a logistic regression model, age (odds ratio (OR), (95% CI): 0.92 (0.92-0.98), p=0.001), burning skin (OR; 3.7 (1.3-10.2), p=0.012) and elevated sICAM-1 (>298ng/mL: OR; 7.4 (2.1-26.0), p=0.002) were independent predictors of hemorrhage. We asked whether sICAM-1 improved prediction by comparing the c-indices from logistic regression models of clinical parameters with or without sICAM-1. The model with sICAM-1 had superior predictive ability (c-index of 0.83 vs. 0.74, p=0.013 by Delong et al.). Using a classification approach (CRT), elevated sICAM-1 (>300ng/mL) followed by age (<35 years) were the best predictors of progression to DHF. In conclusion, these data suggest sICAM-1 may be a clinically useful biomarker to predict complications in dengue fever and may point to endothelial activation as a critical pathway in dengue pathogenesis.

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INTRAHEPATIC INFILTRATING CELLS CAUSE LIVER CELL DEATH IN DENGUE VIRUS INFECTION

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Lymphocyte activation, hepatic infiltration and elevated liver enzyme levels are observed in dengue patients. However, the pathogenic mechanism of liver damage has not been carefully studied. The aim of this study was to investigate the pathogenic mechanism of liver injury in dengue. We have previously shown that immunocompetent C57BL/6 mice infected intravenously by dengue virus have transient elevation of liver enzymes AST and ALT. Employing this model we investigated intrahepatic cellular infiltration and its relationship to liver cell death. Our results showed that dengue virus infection induced CXCL9 and CXCL10 expression in the liver. There was a peak of NK cell infiltration at day 1 after infection. Depleting NK cells reduced the cleaved-form of caspase 3 and the number of $TUNEL^+$ cells in the liver. In addition, following the expression of CCL5, $CD4^+$ T and $CD8^+$ T cell infiltration peaked at day 5 and the infiltrating cells were cytotoxic against dengue virus-infected Hepa 1-6 targets. TCRβ deficiency or $CD8^+$ T cell depletion reduced cleaved-form of caspase-3 and $TUNEL^+$ cells in the liver. Interestingly, intrahepatic $CD8^+$ T cells as well as splenic $CD8^+$ T cells recognized DENV $NS4B_{99}$ as a dominant peptide. These results together show that liver cell death at early time point after infection is related to NK cell infiltration and that at later time point is caused by specific $CD8^+$ T cells cytotoxicity, possibly those that recognize $NS4B_{99}$.

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TREATMENT GUIDED BY RAPID DIAGNOSTIC TESTS FOR MALARIA IN TANZANIAN CHILDREN; SAFETY AND TREATABLE ALTERNATIVE DIAGNOSES TO MALARIA

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WHO guidelines for the treatment of young children with suspected malaria have recently changed from presumptive treatment for malaria to anti-malarial treatment guided by a blood slide or malaria rapid diagnostic

test (RDT). However, there is limited evidence of the safety of this policy in routine outpatient settings in Africa. Children 3-59 months with a non-severe febrile illness and no obvious cause were enrolled over 1 year period in a malaria endemic area of Tanzania. Treatment was determined by the results of a clinical examination and RDT, and blood culture and serum lactates were also collected. RDT-negative children were followed up over 14 days. Overall, 965 children were enrolled; 158(16.4%) were RDT-positive and treated with artemether-lumefantrine (ALu) and 807(83.4%) were RDT-negative and treated with non anti-malarial medicines. Compared with RDT-positives, RDT-negative children were on average younger with a lower axillary temperature and more likely to have a history of cough or difficulty in breathing. Six (0.6%) children became RDT-positive after enrolment, all of whom were PCR-negative for *P. falciparum* DNA at enrolment. In addition, 12 (1.2%) children were admitted to hospital, one with possible malaria, none of whom died. A bacterial pathogen was identified in 9/965 (0.9%) children, 8 of whom were RDT-negative and one was RDT-positive but slide-negative. Excluding 3 children with *S. typhi*, all of the children with bacteraemia were ≤12 months of age and had sensitivity to locally available antibiotics. Compared to double-read research slide results RDTs had a sensitivity of 97.8% (95%CI 96.9-98.7) and specificity of 96.3% (95%CI 96.3-98.4). Use of RDT's to direct the use of anti-malarial drugs in young children did not result in any missed diagnoses of malaria although new infections soon after a consultation with a negative RDT result may undermine confidence in results. Invasive bacterial disease is uncommon in children with non-severe illness and most cases occurred in infants with a current fever.

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REDUCTIONS IN ARTEMISININ-BASED COMBINATION THERAPY CONSUMPTION FOLLOWING THE NATIONWIDE SCALE UP OF ROUTINE MALARIA RAPID DIAGNOSTIC TESTING IN ZAMBIA

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Malaria remains one of the primary reported public health problems in Zambia. To strengthen malaria diagnostic accuracy and thereby reduce the cost associated with treating misdiagnosed individuals with ACTs, the Zambian national malaria control program introduced rapid diagnostic tests (RDTs) for *Plasmodium falciparum*. Zambian policy now recommends that the use of parasite-based diagnosis for every suspected malaria case regardless of age group. However, parasite-based diagnosis is of limited use unless clinicians maintain high adherence to results in subsequent management. A review of facility records was conducted for the period of 2004-2009 in three districts of Zambia; a total of 25 facilities had usable records and were included in the analysis. Median rollout of RDTs at the individual facility level occurred in the first month of 2007. Descriptive analysis showed substantial reductions in the use of ACTs as RDT availability was scaled up. The average proportion of out-patient department attendees treated with an ACT was significantly lower after initiation of RDT testing compared to the pre-RDT period (4.8% vs. 12.9%, p=0.001). By 2009, the number of ACT treatments provided essentially matched the number of RDT positive tests. Multivariate modeling using negative binomial and Poisson regression is being conducted to control for climate variability, vector control coverage, stock-outs of drugs and diagnostics and other potential confounders. Contrary to observations elsewhere in which impact on drug management has been poor, the implementation of RDT-based diagnosis in Zambia appears to be reducing and rationalizing the usage of ACTs even after controlling for a general downward trend in the malaria burden, and may thereby reduce ACT procurement costs while providing more accurate data on disease trends.

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PARASITE-BASED DIAGNOSIS OF MALARIA AMONG SYMPTOMATIC PREGNANT WOMEN IN A TERTIARY HOSPITAL IN AN ENDEMIC AREA

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Because malaria during pregnancy is often asymptomatic in malaria endemic areas, a high index of suspicion is maintained and pregnant women are placed on IPT and also treated presumptively at the earliest suspicion. However, the WHO now recommends that malaria diagnosis be parasite-based as much as possible. We evaluated the prevalence of malaria parasitemia among pregnant women suspected of having malaria by microscopy and Paracheck™ (a histidine-rich protein-2 based malaria rapid diagnostic test, Orchid Biomedical Systems, Goa India) in the antenatal and emergency obstetrics care setting of the University College Hospital in Ibadan, southwest Nigeria between October 2009 and January 2011. The mean age was 30.8years ± 4.7. The prevalence of malaria parasitemia was 22.8% (170/746) and 24.5% (151/617) by microscopy and Paracheck™ respectively. The geometric mean parasite density was 2,091 (range 40-156,975/µL). HIV positivity rate was 7.8% (58/746) while 32.6% (243/746) of enrollees had received at least one dose of IPT-SP by the time of enrollment. 7.5% (56/688) had a temperature ≥ 37.5^{0}C. Women with a Temperature >37.4˚C were significantly more likely to have malaria parasitemia [p<0.0001]. Fever or a history of fever, headache, vomiting, chills and rigors were significantly positively correlated with malaria parasitemia. Over one third (264/746; 35.3%) admitted to haven had a previous attack of malaria in the index pregnancy before enrollment. 44.5% (109/245) of the cases were diagnosed presumptively while the remainder had microscopic diagnosis. ACTs [87; 33.6%], amodiaquine [84; 32.4%], chloroquine [27/259; 10.4%], sulfadoxine-pyrimethamine [17; 6.5%] and artesunate [11; 4.2%] were the most often mentioned antimalarial drugs used. There was no correlation between the presence of malaria parasite and parity or IPT use. The overall sensitivity and specificity of Paracheck were 69.9% and 88.2% respectively while at parasite densities ≥200/µL were 84.8% and 88.7% respectively. Positive predictive value was 66.9% while the negative predictive values for the two cut off parasite densities were 91.1% and 96.3% respectively. In conclusion, presumptive diagnosis of malaria in pregnancy will lead to over treatment. Parasite-based diagnosis of malaria during pregnancy is recommended.

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DEVELOPMENT OF A RAPID DIAGNOSTIC TEST (RDT) FOR SIMULTANEOUS DETECTION OF G6PD DEFICIENCY AND MALARIA INFECTION

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G6PD deficiency is the most common human enzyme deficiency affecting 400 million people and highly prevalent in malaria endemic areas. This deficiency appears to provide some protection from malaria infection, but it can also cause hemolysis after administration of some malarial drugs, especially primaquine. There is an urgent need of rapid diagnostic test (RDT) suitable for the field to screen glucose 6 phosphate dehydrogenase (G6PD) deficiency before treatment with malarial drugs. We have developed a rapid test in dry format assay for the qualitative detection of G6PD enzyme activity. The G6PD RDT test result using clinical samples correlated with the quantitative test results and detected around ≤4 IU/Hg (normal 12± 2.09 at 37 ˚C) as deficient. The cutoff will be adjusted

depending on the tolerance of red blood cells against primaquine in G6PD deficient patient. Accelerated stability studies showed that test strips were stable for 2 months at 45°C and 10 days at 60˚C. This G6PD test RDT can be combined with a malaria RDT which is capable of detectings less than 30 parasites per µl of blood and is stable for 2 years at 40˚C as a dual test. This dual test kit diagnoses malaria infection and G6PD deficiency at the same time using small amount of blood (less than 10 µl). The test is rapid (<10 min), simple to use, inexpensive, portable, and has no special storage requirement which is critical element for field use.

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DRIED *PLASMODIUM FALCIPARUM* POSITIVE BLOOD AS QUALITY CONTROL SAMPLES FOR FIELD MONITORING OF MALARIA RDT PERFORMANCE

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Malaria rapid diagnostic tests (RDTs) are critical to the WHO recommendation for parasitologic confirmation of all suspected malaria cases. RDT performance is compromised by the high temperature conditions typical of malaria endemic regions. Despite this limitation, methods to monitor RDT performance in the field after exposure to such conditions are lacking. Positive control samples are unavailable and comparing RDTs to blood smears is not ideal since RDTs detect parasite antigens and microscopy detects parasites. Furthermore, accurate slide reading requires expertise unavailable in most health facilities. Currently, no reliable quality control (QC) method exists for RDTs in the field. We determined the suitability of dried parasite positive blood as QC samples for malaria RDTs using 3 *Plasmodium falciparum* culture strains at 200 and 2000 parasites/µl (p/µl) on 10 high-performing (WHO/FIND Evaluation) RDT brands. After baseline testing, 50µl aliquots of parasite positive blood were air-dried overnight, stored at 35˚C, 25˚C or 4˚C for 1, 4 and 12 wks and then tested on the same RDTs after rehydration with PBS-tween. All dried blood at 2000p/µl retained reactivity (100% sensitivity) on all 10 RDT brands at all temperatures and times points for HRP2. At 2000p/µl, sensitivity on the pLDH (Pan) bands for 2 Combo tests was reduced to 80-89%. Dry blood at 200p/µl for all storage temperatures and time points were detected at 100% sensitivity on 6 of 10 RDTs for HRP2. The sensitivity on 2 of the 4 remaining RDTs was 100% up to 4 weeks of storage at all temperatures, dropping to 87.5% at wk 12 for wk 12 samples stored at 35˚C. Sensitivity of detection on pLDH bands was low (29%, range 0-100%) at 200p/µl; partly attributable to weak baseline reactivity, and thus RDT brand. In the absence of positive control antigens, well-characterized parasite positive dried blood can be successfully used as a simple tool for monitoring RDT, especially HRP2 test performance in the field. The sample used should be well characterized for its baseline reactivity on the RDT it is intended to monitor.

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GOING BELOW THE TIP OF THE ICEBERG: DOES MOLECULAR DETECTION OF MALARIA CHANGE OUR UNDERSTANDING OF EPIDEMIOLOGY AND CONTROL?

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Molecular detection of *Plasmodium falciparum* malaria by PCR-based techniques has revealed low density infections in individuals who would previously have been classified as uninfected by standard microscopy, fundamentally changing estimates of malaria infection prevalence. However, the relevance of submicroscopic cases to control programmes remains unclear. Here we develop a quantitative framework for describing the distribution of low density infections and discuss malaria epidemiology and control in light of these data. We analysed 114 population surveys in which *P. falciparum* prevalence was measured by both microscopy and PCR, allowing for measurement error with Bayesian methods. In line with previous results, we find a significant increase in the sensitivity of microscopy as the underlying transmission intensity increases, from 60% when PCR prevalence is over 80%. We explore a number of hypotheses which could generate this observed relationship between microscopy and PCR prevalence using mathematical models, and find that it can be captured by variation across endemicities in a combination of the following (1) average age of malaria infections, (2) rates of partially successful treatment, (3) PCR contamination and (4) genetic diversity of parasites. We find that PCR prevalence can be estimated relatively accurately from microscopy prevalence with the best-fitting model giving a 91% correlation between predictions and data. Combining our analysis with two available estimates of the infectiousness of submicroscopic infections to mosquitoes, our results suggest that their contribution to the infectious reservoir may be important mainly in low transmission settings. Where slide-prevalence is 50% of mosquito infections versus <10% where slide-prevalence is >60%. Therefore PCR detection is likely to be worthwhile in areas with low transmission, for example during active case detection programmes, but of less concern to control agencies in more highly endemic areas.

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WITHIN HOST AND WITHIN COMMUNITY POPULATION DIVERSITY OF *PLASMODIUM FALCIPARUM CSP* T CELL EPITOPES BY MASSIVELY PARALLEL PYROSEQUENCING IN LILONGWE, MALAWI

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Plasmodium falciparum circumsporozoite surface protein (*csp*) is the current leading candidate for a malaria vaccine with the RTS,S vaccine in a phase III clinical trial. Immunity appears to be mediated both through antibody responses and T cell responses. The C-terminal region of *csp* contains two T cell epitopes (Th2 and Th3) which are highly polymorphic. Genetic diversity of the parasite may in the long run affect the ability of any vaccine to prevent disease. Parasite genetic diversity within individuals and within communities is not adequately described by traditional genotyping methods because they fail to capture all variants. To better describe the diversity of the *csp* T cell epitopes in Lilongwe, Malawi, we employed massively-parallel pyrosequencing (Roche 454 System) to sequence a 319bp amplicon of *csp* containing the Th2 and Th3 epitopes from 100 participants with uncomplicated malaria. Over 470,000 sequences aligned to the region of interest, of which about 360,000 were used to generate haplotypes and frequencies. We detected over 80 unique genetic haplotypes in the population. The average multiplicity of infection was 3.4 variants (range: 1-16). Using population genetic analysis methods, we estimated the total number of genetic haplotypes within the community and compared the diversity of parasite populations between adults and kids. The combination of ultra-deep sequencing and population genetic analysis provides tools to study and exploit the genomic diversity of *P. falciparum* within individual infections and within communities.

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A NOVEL ASSAY FOR IDENTIFYING GAMETOCYTOCIDAL COMPOUNDS AGAINST *PLASMODIUM FALCIPARUM*

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The apicomplexan parasite *Plasmodium falciparum* that causes malaria, still manages to kill up to 1 million people a year. Most of these deaths

occur in sub-Saharan African, where cheap and effective drugs are not always available or used efficaciously. In order to eradicate malaria it will be necessary to find drugs that can effectively kill all of the erythrocytic stages of the parasite, including gametocytes. Gametocytes are the only stage that leads to transmission of the disease from the human host to the mosquito vector. Gametocytes are difficult to study as unlike the asexual stages of the parasite they do not replicate in culture, as they are terminally differentiated. In order to find effective anti-transmission blocking drugs for malaria a method was required to produce sufficient gametocytes to be able to conduct drug assays. Gametocytes do not synthesise hypoxanthine, so a novel method of determining parasitic growth inhibition was also needed. Here we report on a novel assay method that enables drugs and compounds to be tested and novel gametocytocidal compounds to be identified. A method has been devised that allows large numbers of gametocytes to be produced and used in an assay that measures ATP production. The assay measures the number of live gametocytes after drug treatment and hence the gametocytocidal activity of compounds. The assay was tested against a small blinded library of compounds to provide a proof of concept. The ATP assay was able to identify a small number of compounds with previously known gametocytocidal activity and provide IC_{50}s against late stage gametocytes, for these compounds. The assay is now being optimised for high throughput screening to provide the first HTS assay for gametocytocidal compounds. This research has provided the first step in being able to identify drugs that will block the transmission of malaria and aid in the eventual eradication of this disease.

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VERY LONG LASTING EFFECT OF INTRA-MUSCULAR DECOQUINATE IN THE *PLASMODIUM CYNOMOLGI BASTIANELLII* (B STRAIN) RELAPSING MALARIA MONKEY MODEL

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Global eradication of malaria will require simple, safe treatment regimens to eliminate the persistent liver stage associated with *Plasmodium vivax* and *P. ovale*. Currently the only FDA approved treatment for this indication is primaquine. Unfortunately, the 8-aminoquinoline (8AQ) class is associated with hemolytic anemia in persons deficient in glucose-6-phosphate dehydrogenase (G6PD) necessitating the need for new drugs that are effective against hypnozoites without the G6PD liability. A number of non-8AQ compounds have been reported to possess anti-hypnozoite activity against sporozoite-induced *P. cynomolgi B* infections in Rhesus monkeys, including the quinoline esters WR197236 and WR194905. However, radical cures eliminating dormant hypnozoites were achieved only when the compounds were injected intramuscularly (IM). Decoquinate (DQ), a coccidiostat approved by the FDA for use in veterinary medicine, is structurally similar to WR194905 and if effective against hypnozoites could be pursued as a non-8AQ option. When co-administered with chloroquine (CQ), DQ cleared relapsing *P. cynomolgi* parasites from two infected Rhesus monkeys for over 100 days, a positive test for radical cure as established in the model (DQ: 15 mg/kg IM daily for 7 days, CQ: 10 mg base/kg oral daily for 7 days). Upon extended monitoring, however, relapsing parasitemia was observed well over one year after the initial clearance (days 509 and 511 after the last day of drug dosed). Two monkeys administered double the aforementioned dose of DQ (30 mg/kg IM once daily for 7 days) remained clear of parasitemia at this time. When DQ was co-administered orally (either 20 or 40 mg/kg BID for 14 days) with CQ, only one monkey in the high dose group showed any delay in parasitemia when compared to the purely anti-erythrocytic CQ controls. These results strongly suggest that the absence of relapse in the higher IM DQ dose is not a true radical cure, *i.e.* - elimination of all stages of parasites, but rather parasite suppression by long-lasting circulating drug levels from an IM depot effect.

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ESTABLISHMENT OF A RHESUS MONKEY MODEL TO PREDICT G6PD DEFICIENCY-RELATED HEMOLYTIC POTENTIAL FOR ANTIMALARIAL DRUG DEVELOPMENT

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Primaquine (PQ), an 8-aminoquinoline (8-AQ), is used for radical cure of *Plasmodium vivax* and to kill stage V *P. falciparum* gametocytes. However, this drug often triggers hemolytic anemia in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. G6PD and glutathione (GSH) are known to protect red blood cells (RBCs) from oxidative damage leading to hemolysis. A suitable animal model is needed to screen new prospective nonhemolytic compounds. In this project, we sought to establish a nonhuman primate (NHP) model to closely mimic the hemolysis caused by PQ treatment in the G6PD deficient subjects. We hypothesized that GSH depletion of rhesus monkey RBCs (rmRBCs) *in vitro* and infusion of the GSH-depleted RBCs back into the animals could result in accelerated clearance of rmRBCs similar to hemolysis associated with PQ in human G6PD subjects. Blood was drawn from monkeys and subsequently treated with diethyl maleate and buthionine sulfoximine *in vitro* to deplete GSH to a residual level of 5-15 % and were labeled with a green fluorescent dye at the same time. The cells were then infused back to the original donors. PQ or other antimalarial drugs, i.e., tafenoquine (TQ), chloroquine (CQ), or mefloquine (MQ), were orally administered once a day immediately after the infusion. The blood samples were collected daily to monitor the fate of the RBCs by flow cytometry. PQ, either at 4 or 1.78 mg/kg, significantly accelerated clearance of GSH-depleted RBCs in a time-dependent manner and high dose TQ (6 mg/kg) also showed a significant acceleration of rmRBC clearance in comparison to control animals. In contrast, treatment with nonhemolytic drugs, CQ (25 mg/kg) and MQ (25 mg/kg), did not result in any accelerated rmRBC clearance. These findings clearly support our hypothesis that the artificially GSH-depleted rmRBCs mimic hemolysis seen in G6PD deficient patients treated with PQ. A NHP model is now available for antimalarial drug development and for identification of combinations to improve the therapeutic index of existing drugs.

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ENANTIOSELECTIVE METABOLISM AND PHARMACOKINETICS OF PRIMAQUINE IN PRIMARY HUMAN HEPATOCYTES, MICE AND NORMAL HUMAN VOLUNTEERS

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Primaquine (PQ) is clinically used in a racemic form. Earlier studies have indicated differential therapeutic profiles of PQ enantiomers. Enantioselective pharmacokinetic (PK) and pharmacologic characteristics may contribute to this property. Carboxy PQ (cPQ) has been demonstrated as a major plasma metabolite in animals as well as humans. Rate of

formation of cPQ could be a primary determinant of the pharmacologic/toxicological responses to this drug. Studies were undertaken to investigate enantioselective metabolism and PK of PQ *in vivo* in mice, normal human volunteers and *in vitro* in primary human hepatocytes. A chiral LC-MSD-TOF method for simultaneous analysis of enantiomers of PQ and cPQ was employed. In a PK study in mice administered a single 30 mg/kg dose of racemic PQ, (+) and (-)PQ exhibited similar plasma PK profile. However plasma (-)PQ level declined more rapidly compared to (+) PQ. A pronounced difference was noted in the plasma PK profile of cPQ enantiomers. The Cmax for (-)cPQ was >10 fold higher than (+)cPQ. A study conducted in normal human volunteers, after a single oral dose of 45 mg racemic PQ, confirmed differential PK profiles of PQ enantiomers. The plasma cPQ peaked at about 8 hours after drug administration and remained elevated for 24 hours; all of the plasma cPQ was due to the (-)cPQ, and (+)cPQ levels were under the detection limits, while plasma levels of both (+) and (-)PQ were low and variable throughout the study period. Primary human hepatocytes also differentially metabolized the PQ enantiomers. (-)PQ was more rapidly metabolized to (-)cPQ as compared to (+)PQ. Inhibitor studies suggested a major role of monoamine oxidase-A for formation of cPQ. These studies confirm that the two enantiomers of PQ have differential metabolic and PK profiles, which suggests they likely will have different efficacy and toxicity profiles. The studies strongly support further clinical evaluation to find if one enantiomer will afford a better therapeutic value over another.

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EFFECT OF CYP 3A4 INHIBITORS ON THE HEMATOLOGICAL TOXICITY AND PHARMACOKINETICS OF AN 8-AMINOQUINOLINE DEVELOPMENTAL CANDIDATE, NPC1161B

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8-Aminoquinoline antimalarial drugs (8-AQs) have broad utility and excellent efficacy, but also have limitations due to hematological toxicity in subjects with glucose-6-phosphate dehydrogenase deficiency (G6PDd). We previously showed that NPC1161B (NPCB), the (-) (*R*) enantiomer of (±) (*RS*)-8-[(4-amino-1-methylbutyl)amino]-6-methoxy-4-methyl-5-[3,4-dichlorophenoxy] quinoline succinate, proved extremely efficacious in animal models of malaria and leishmania. *In vitro* studies suggested that multiple human cytochrome P450 (CYP) isoforms mediate the generation of methemoglobin (mtHb) in the presence of the (+) enantiomer; however, for the (-) enantiomer (NPCB), CYP3A4 was the major contributor. The present studies assessed whether CYP3A4 inhibitors could block the hematological responses of NPCB in dogs. In one study, ketoconazole (KTZ) was administered p.o. (200 mg/kg), at 1 hr before and 11 and 23 hr after, a single oral dose of NPCB at 15 mg/kg. NPC caused a sustained rise in mtHb, from baseline of 1% to peak at 10% on d 6, with a slow decline, remaining elevated at 8% on d 12. KTZ administration inhibited the rise in mtHb (3% at d 6 and 4% at d 12). KTZ increased plasma levels of NPCB by about 50%. In the next study, NPCB was given at 15 mg/kg/d p.o. on days 1-4, and ritonavir (RTN) was given at 10 mg/kg p.o. twice a day on days 1-8. A marked increase in mtHb (peak at around 20% on day 8-10) was observed in the absence of RTN, with an increase in reticulocytes (RET) and nucleated red blood cells (nRBC). RTN completely prevented the rise in mtHb, RET and nRBC, suggesting that the hematological changes are indeed dependent on a CYP3A4-mediated pathway. However, unexpectedly, it was observed that RTN reduced peak plasma levels and AUC for NPCB by 55%, likely due to decreased intestinal absorption of NPCB in the presence of RTN. In conclusion, two CYP3A4 inhibitors reduced the metHb formation and erythropoiesis after high doses of NPCB. However, their effects on pharmacokinetics of the drug were divergent, and other inhibitors should be evaluated.

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OZ439 IN MALARIA PATIENTS, PRELIMINARY RESULTS FROM A PROOF OF CONCEPT STUDY IN ADULTS WITH *PLASMODIUM FALCIPARUM* OR *P. VIVAX* INFECTION

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OZ439 is a synthetic endoperoxide (1,2,4-trioxolane) which shows great promise in preclinical studies as part of a single dose cure for uncomplicated malaria. *In vitro*, it kills the parasite with a similar parasite reduction rate as artemisinin, and in phase I studies, a plasma concentration above the IC_{90} can be maintained for >72 hours. We have studied OZ439 in an open-label, phase IIa trial in 60 patients (two groups of 10 patients per dose cohort) with either uncomplicated *Plasmodium vivax* or *P. falciparum* malaria mono-infection (ClinicalTrials.gov: NCT01213966). After enrolment, patients received a single dose of OZ439. Patients were monitored for parasite load and pharmacokinetics every 6 hours, and standard of care treatment was administered between 36 and 72h. The primary efficacy endpoint was parasite reduction rate (PRR). OZ439 was well tolerated. Contrary to the results we obtained with a previous endoperoxide, OZ277 (Rbx11160), the plasma exposure of OZ439 was similar to that seen in volunteers. OZ439 was fast acting, with a Parasite Reduction Rate comparable to the PRR of three day artesunate treatment in hyperparasitemic patients. These data support a key role for OZ439 as one component of a new combination single dose cure for malaria.

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EFFICACY OF A TWO- VERSUS THREE-DAY COURSE OF DIHYDROARTEMISININ-PIPERAQUINE IN NORTHERN CAMBODIA: RESULTS FROM AN OPEN-LABEL, RANDOMIZED CLINICAL TRIAL

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Declining efficacy of artesunate-mefloquine has prompted a recent change to dihydroartemisinin-piperaquine (DP) as the first line therapy against all malaria infections in Cambodia. Although 3 days of DP

therapy is recommended in the civilian sector, a 2-day regimen of DP is currently used by the Cambodian military to improve compliance. As part of an active observational malaria epidemiology cohort study in Oddar Meancheay Province, an area of high transmission in northern Cambodia, 200 healthy volunteers were enrolled and followed weekly for up to 4 months. All subjects developing uncomplicated malaria were randomized to receive directly observed therapy with 320/2880 mg of DHA-piperaquine given over 2 or 3 days (n = 40 per arm). Subjects were followed weekly for a minimum of 42 days and assessed for treatment efficacy, safety and tolerability. The trial was powered (80%) to detect an expected 25% higher recurrence rate in the 2-day group compared to 3 days. From September 2010 to February 2011, 80 malaria patients were randomized to DP, 16 (20%) with *P. falciparum*, 61 (76%) with *P. vivax* and 3 (4%) with mixed infection. PCR-uncorrected per protocol 42-day efficacy rates against all malaria species combined were not statistically significantly different between treatment groups: 89% (95% CI 76-96%) for 2 days and 92% (95% CI 80-97%) for 3 days. Intention to treat efficacy rates were also not significantly different: 83% (95% CI 68-91%) for 2 days and 88% (95% CI 74-95%) for 3 days. Median parasite clearance times were 11.1 hours for *Plasmodium vivax*, but 72.5 hours for *P. falciparum*; there were no significant differences between treatment groups. DP was safe and well tolerated without significant treatment-related adverse events. PCR uncorrected all-malaria efficacy was not significantly different between 2 and 3 days of DP in this population on the Thai-Cambodian border. However, 42-day cure rates appear to be lower than previously reported. Given the proximity of this study site to areas of known multi-drug resistance this finding is concerning.

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CHROMOSOMAL INTEGRATION OF TRANSGENES AND DERIVATION OF A STABLE TRANSGENIC LINE IN THE PARASITIC NEMATODE *STRONGYLOIDES RATTI*

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Parasitic nematode infections adversely affect over one billion people. Genetic transformation is a potential tool for analyzing gene function to identify new drug and vaccine targets in these worms. We have developed a robust system for transgenesis in *Strongyloides* spp. using gonadal microinjection for gene transfer. Using this system, transgenes are expressed in promoter-regulated fashion in the F1 but are silenced in subsequent generations, presumably because of their location in repetitive episomal arrays. To counteract this silencing, we explored transposon-mediated chromosomal integration of transgenes in *S. ratti*. To this end, we constructed a donor vector encoding green fluorescent protein (GFP) under the control of the *Ss-act*-2 promoter with flanking inverted tandem repeats specific for the *piggyBac* transposon. Free-living *Strongyloides ratti* females were transformed with this donor vector and a helper plasmid encoding the *piggyBac* transposase. The transgene was detected in the F1 and later generations by PCR, and 15.8% of F1 larvae were GFP-positive. We inoculated a rat with 34 F1 GFP-positive infective larvae (L3i), and 0.48% of 6014 F2 individuals resulting from this host passage expressed GFP. We cultured GFP-positive F2 individuals to produce GFP-positive F3 L3i for additional rounds of host and culture passage. GFP expression frequencies in subsequent generations were 74.24% in F3, 98.99% in F4, 82.39% in F5 and 100% in F6. The resulting transgenic line now has uniform GFP expression among all progeny. Chromosomal integration of the reporter transgene in *S. ratti* was confirmed by Splinkerette PCR, which revealed the transgene flanked by *S. ratti* genomic sequences corresponding to at least three discrete integration sites. BLAST searches of flanking sequences against the *S. ratti* genome revealed integrations in three contigs: 75336 (position 3211), 74996 (position 155901) and 74278 (position 172601). This result provides the basis for two powerful functional genomic tools in *S. ratti*: hertiable transgenesis and insertional mutagenesis.

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NEOMYCIN SELECTION OF TRANSGENIC *SCHISTOSOMA MANSONI*

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Draft genome sequences for *Schistosoma mansoni* and *S. japonicum* were reported recently, a landmark event that ushered in the post-genomic era for schistosomiasis. Analysis of target genes to underpin new interventions for schistosomiasis requires functional genomics approaches such transgenesis that will validate essential genes to be targeted with drugs or vaccines. We have adapted murine leukemia retrovirus (MLV) vectors -widely used in human gene therapy research- to transduce schistosomes, leading to integration of reporter transgenes into the parasite genome. Drug selection of transgenic schistosomes would be highly desirable in order to provide a means to enrich for populations of transgenic worms in virion-exposed parasites. Given that *neoR* (the gene encoding resistance to neomycin/G418) driven by the MLV's 5'-LTR as promoter is actively expressed in schistosome tissues, and that G418 is lethal under the conditions tested here, we investigated whether MLV transduced schistosomules could be rescued on G418. First, a dose-response kill curve and lethal G418 concentrations were established. Second, one day old schistosomules were infected with MLV at two concentrations of virions, 1X and 3X. Transduced worms were cultured with or without G418 and by day 10, aliquots of schistosomules from the groups were stained for viability with Trypan blue and enumerated. No significant differences were observed among the group of parasites without G418. However, significant differences were found among schistosomules cultured with G418 where more schistosomes survived when transduced with virions (3x) in comparison to controls (p=0.0039). Remarkably, *neoR* expression levels in the group subjected to G418 selection was higher than in worms treated with the same titer of virus but cultured without G418. This likely reflects enrichment of transgenic schistosomules within the population of transduced parasites subjected to G418 pressure. This appears to be the first report of antibiotic selection of transgenic schistosomes or indeed of any transgenic helminth parasite species.

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THE COMPLETE *WOLBACHIA* GENOME AND TRANSCRIPTOME FROM *ONCHOCERCA OCHENGI* INDICATES A DIFFERENT WORM-SYMBIONT RELATIONSHIP TO THAT OF *BRUGIA MALAYI*

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The filarial nematode *Onchocerca ochengi*, a parasite of cattle, is recognised as the closest relative of *O. volvulus*, the aetiological agent of human onchocerciasis. In common with the filariae that cause lymphatic filariasis (including *Brugia malayi*), *O. ochengi* and *O. volvulus* contain *Wolbachia* endobacteria in the hypodermal cords of both sexes and in the reproductive tract of female worms. *Wolbachia*, which are much more prevalent in arthropods than in nematodes, are divided into approximately 10 supergroups. Four complete *Wolbachia* genomes have been published to date: two in supergroup A (from *Drosophila* spp. hosts), one in supergroup B (from a mosquito host), and one in supergroup D (strain *w*Bm from *B. malayi*). Here, we report the first complete genome of a supergroup C *Wolbachia* (strain *w*Oo from *O. ochengi*), alongside complete endobacterial transcriptomes obtained by deep sequencing of cDNA from both hypodermal cord and female reproductive tract tissues. At 0.96 Mb, the *w*Oo genome is the smallest thus far characterised for any *Wolbachia* and is 11% smaller than that of *w*Bm. In contrast to *w*Bm,