orthodox, delay in providing accurate diagnosis, appropriate treatment and prompt referral. 4) The fourth delay was within the competent hospital or health facility due to attitude of health workers, and logistics in releasing test results and initiating treatment. Fifty-five % had a delay of over 3 months while 28% had delays of over 6 months. About 71.5% of patients had received inappropriate care in peripheral centers before commencement of appropriate TB therapy. In conclusion, delay in seeking care is a prevailing issue in TB control in Nigeria TB control efforts should target delays through appropriate health education messages both to the community and health providers for early and prompt report, diagnosis and referral to competent TB centers.

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USE OF MALARIA PREVENTIVE MEASURES IN PREGNANCY AND PLACENTAL/NEONATAL PARASITAEMIA: A MULTICENTRE BASELINE EVALUATION IN NIGERIA

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Malaria in pregnancy has been a topical issue in recent times and most of its effects in newborns have been widely reported in the literature except for congenital malaria. This was previously reported to be very rare but has been increasingly reported in recent times in Nigeria, although based on results from small population studies. Clinicians have found this situation very worrisome, thus raising the need to examine the existing practices in the country in relation to the use of malaria preventive measures in pregnancy. This study was undertaken to evaluate on a large scale the current status of use of malaria preventive measures (anti-vector measures, chemoprophylaxis, and intermittent presumptive treatment with sulphadoxine-pyrimethamine (IPTp-SP) and their relationship with prevalence of placental and congenital malaria in Nigeria. This was a multi-centre prospective and descriptive study conducted between April 2003 and March 2004. Two thousand five hundred mother infant pairs were recruited from four geopolitical zones with varying epidemiological characteristics regarding malaria. The majority of babies (89.8%) were delivered at term and only 6.1% had low birth weight. Proportion of use of malaria preventive measures were: mosquito screens (44.8%), insecticide sprays (35.5%) while only 2.5% used insecticide treated nets. Overall, 88.5% of the mothers received some form of medications for malaria prevention comprising of pyrimethamine (76.8%), proguanil (1%), IPTp-SP (11.7%), Chloroquine (4%). A small proportion (0.1%) of the women used herbs. Babies of mothers on IPT-Sp were least likely to have parasitemia (2%) (P<0.05). The combination of chemoprophylaxis/IPTp-SP and anti-vector agents was found to be most effective in reducing parasitemia in the newborns (X² = 5.85, P=0.016).

Given the efficacy of IPTp-SP in this study and others done elsewhere in sub-Saharan Africa, there is a need to promote the use of IPTp-SP throughout Nigeria and its combination with anti-vector agents should be emphasized. The campaign to promote the use of insecticide treated nets for prevention of malaria in pregnancy should also be intensified in Nigeria as the usage is still very low.

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PERIPARTUM MALARIA IN NIGERIA: CURRENT STATUS AND PREGNANCY OUTCOME

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Malaria in pregnancy is an important cause of a variety of maternal and perinatal adverse consequences. In Nigeria, several studies focusing on malaria in the peripartum period have been conducted but with variable findings that has mitigated against a focused policy on prevention. This study was undertaken to evaluate the current status of peripartum malaria and its impact on neonatal outcome in Nigeria. A descriptive study was conducted in four centers located in four different geographical zones in Nigeria over a 12-month period. Focused clinical and laboratory examination were done in the subjects within 2 hours of parturition. Maternal peripheral and placental blood samples were taken for determination of malaria parasitaemia using Giemsa stain of the thick and thin blood films. Maternal and baby’s haematocrit were also taken at birth. There was a quality assurance procedure for the study. A total of 2500 subjects were recruited. A total of 625 of the data sets were excluded due to breach in the study protocol. The women were aged between 14-50 years. The proportion of preterm deliveries was 10%. There were a total of 404 positive smears, giving a peripartum prevalence of malaria of 21%. Those with positive peripheral smears were 319 (17%) while those with positive placental smears were 267 (14.2%). There was a significant decrease in proportion of women with placental parasitaemia with increasing parity (p=0.04). Maternal age less than 20 years was significantly associated with both peripheral (p=0.016; OR=2.3; CI=2.4.9) and placental parasitaemia (p=0.01; OR=2.6; 1.2-5.4). However after adjusting for covariates only the age of the women <20 years was significantly associated placental parasitaemia. Peripheral parasitaemia in the women was associated with a lower mean haematocrit 0.34±0.5 versus 0.37±0.54 (p=0.001); lower mean birth weight (p=0.001); and a significantly higher proportion of low birth weight babies: 24.2% versus 16.5% (p=0.025; OR=1.65 [1.12-5]). In conclusion, in Nigeria 1 in every 5 women have malaria parasitaemia at delivery. Maternal age, less than 20 years, was the most important predisposing factor. Reduction in maternal haematocrit, mean birth weight and a higher proportion of low birth weight babies were the major outcomes of malaria in the peripartum period.

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MALARIA SITUATION: RISK AND CONTROL IN TSUNAMI-AFFECTED AREAS, PHANG NGA PROVINCE, THAILAND

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In December 2004 the Asian tsunami caused more than 200,000 deaths. The WHO and other health organizations warned of possible communicable disease outbreaks in all tsunami affected areas. We
conducted a study comparing malaria incidence in Phang Nga, the coastal Thailand province most severely affected by the tsunami. Relative to the annual pre-tsunami incidence the annual incidence immediately following the tsunami increased in the five inland provincial districts but was stable in the three coastal districts directly sustaining tsunami damage. The increase in the non-tsunami-affected districts might be attributable to an influx of foreign workers, the diversion of malaria control resources from non-tsunami-affected to tsunami-affected districts; or other factors. Interestingly, an entomological survey conducted in a tsunami affected area noted to have a minor malaria outbreak detected the presence of Anopheles sundaicus, a species complex with a predilection for brackish coastal areas. Continued active malaria control measures and long term surveillance are recommended.

AN INVESTIGATION INTO THE POTENTIAL CARDIOTOXIC INTERACTIONS OF QUININE AND ARTEMETHER/ LUMEFANTRINE (COARTEM®) WHEN USED SEQUENTIALLY IN THE TREATMENT OF MALAWIAN CHILDREN WITH SEVERE MALARIAL ANEMIA

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Severe malaria is a major cause of childhood morbidity and mortality in African children. Currently, the common treatment is parenteral quinine followed by oral sulfadoxine/pyrimethamine (SP). Due to fast development of SP resistance many African countries are in the process of replacing SP with artemether/lumefantrine (Coartem®). Lumefantrine is structurally related to quinine, which is known to give marked QTc interval prolongation on electrocardiogram (ECG). Studies so far have shown that Coartem alone does not cause clinically relevant QTc prolongation. But, the potentially important cardiotoxic consequences of sequential quinine and Coartem treatment in children have not been studied. We have evaluation, in an open labeled study, ECG changes in 42 Malawian children who received parenteral quinine followed by Coartem as part of their severe malaria anemia management. The patients received a blood transfusion and were started on parenteral quinine (total of 5 doses) followed by 6 dose Coartem course. A twelve lead ECG was recorded just before the 1st (0 hrs) and 6 hours after the 1st (6 hrs) and 6th (78 hrs) Coartem dose and after 28 days. ECG analysis was done by automated software package and manual reading. One hundred and eight ECG recordings were available for analysis. None of the children, at any time point, had a QTc interval measure of more than 550msec (primary endpoint). QTc intervals of >450 but less then 480msec were found in 2.2%, 4.9%, 7.7% and 8.1% of ECG recordings at 0, 6 and 78 hours, and 28 days respectively. None of the patients was found to have a >60ms increase in QTc interval when compared to base line at any time point. No arrhythmia or syncpe was observed in any of the children. In the first 42 children with severe malaria anemia studied, we did not find an indication of a cardiotoxic effect when quinine and Coartem were given sequentially. This is an encouraging finding, since cardiac toxicity would have posed serious limitations for the use of Coartem in the final stage of antimalarial treatment following a severe malaria episode. In order to improve the power of the study, recruitment is continuing. At the conference ECG findings from a considerably larger study population will be available for presentation.

TOXOPLASMA GONDII INFECTION IN THE UNITED STATES, 1999-2004

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Toxoplasma gondii infection can be responsible for congenital or acquired disease that leads to neurologic and ocular illness. To determine the recent prevalence of T. gondii infection in the U.S. population, we tested sera collected from the National Health and Examination Survey (NHANES) in 1999 through 2004 for T. gondii specific immunoglobulin G antibodies (Platelia Toxo-G EIA, BioRad, Hercules, CA) on persons age 6-49 years. We also compared the results to those obtained in NHANES III (1988-94). Of 18,433 persons 6-49 years old selected and examined in NHANES 1999-2004, 15,960 (90%) had sera tested for T. gondii antibodies. The age-adjusted T. gondii seroprevalence among those 6-49 years old was 10.8% (95% confidence limits [CL] 9.6%, 11.9%), and among women of childbearing age (15-44 years old), 11.0% (95% CL 9.5%, 12.4%). Seroprevalence increased with age; the age-adjusted seroprevalence was higher among persons below the poverty level (14.5%) than those at or above the poverty level (9.9%) (p<0.001), and higher among non-Hispanic black (12.1%) and Mexican American (13.7%) than among non-Hispanic white persons (8.7%) (p=0.01 and p<0.001, respectively). However, among U.S.-born persons age 6-49 years the age-adjusted seroprevalence was lower in Mexican Americans (4.6%) than non-Hispanic blacks (10.4%) or whites (8.1%) (p<0.01 and p<0.001, respectively). When comparing U.S.-born persons in the overlapping 12-49 year age group, from NHANES III (1988-1994) to NHANES 1999-2004 there was a reduction in the age-adjusted T. gondii prevalence from 14.1% to 9.0% (p<0.001). Although T. gondii still infects many persons in the United States, the prevalence has decreased in the past decade.

MALARIA ELIMINATION IN HISPANIOLA: A REALISTIC GOAL?

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Malaria remains a problem in Hispaniola, the Caribbean island shared by Haiti and the Dominican Republic. In Haiti (pop. 8 million), more than 20,000 cases of confirmed malaria were reported in 2005. This statistic is, however, unreliable due to under-reporting and inadequate quality of microscopic diagnosis in many health facilities. Two countrywide health facility surveys in Haiti in 1995 and 2005 showed that, among patients with clinically suspected malaria, 4.0% and 3.4%, respectively, were infected with Plasmodium falciparum. In the Dominican Republic (pop. 9 million), 3,058 cases of confirmed malaria were reported in 2005. In the Dominican Republic, most cases occur in persons aged 10-49 years (74%) and in rural areas (75%); in 2004, 28% of the cases were in Haitian nationals, mostly migrant workers in sugarcane plantations or construction projects. Malaria is concentrated in defined areas of the island, with most cases found in the Haitian departments of Grande Anse, Nippes, Artibonite, and South, and in the Dominican provinces of Bahoruco, Barahona, Azua, and La Altagracia. In addition to its health impact, malaria also affects the economy of the island; the tourism industry in the Dominican Republic reported a loss of 200 million USD after 18 tourists developed malaria in late 2004 following a visit to the coastal resorts of

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Punta Cana and Bavaro. Malaria elimination in Hispaniola is a realistic objective because: a) malaria has been eliminated from all other Caribbean islands; b) malaria in Hispaniola is not highly prevalent and tends to be focal; c) P. falciparum, the only species found in the island, does not relapse and remains chloroquine sensitive to date; and d) the main vector, Anopheles albimanus, is relatively inefficient. To achieve elimination, Haiti and the Dominican Republic need to coordinate their control efforts and adopt jointly a comprehensive package of interventions including surveillance, early treatment of infections, insecticide-treated bednets, treatment of mosquito breeding sites and residual insecticide spraying.

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DETECTION OF LEISHMANIA PARASITES IN AN OUTBREAK SITE IN GHANA USING POLYMERASE CHAIN REACTION

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A recently recognized outbreak of Leishmaniasis in Ghana has prompted research into the epidemiology of the disease in this region. Initial published findings indicated that the causal agent was Leishmania major. To further characterize this outbreak, we used a real time PCR method to identify Leishmania-infected individuals in Ghanaian villages, and have sought to identify the local vector and mammalian reservoir which complete the disease cycle. Our study will encompass the 2006 calendar year during which human, mammalian and sand fly samples will be obtained on a monthly basis from field collections from the outbreak foci around Ho, Volta Region, Ghana. This sampling scheme attempts to elucidate the disease cycle and any seasonality to the outbreak. To date, 88 samples have been assayed including nine tissue samples from humans, 33 rodent tissue samples, 43 tissue and blood samples from domestic animals, and 25 pools of sand flies (~250 flies). Using a primer-probe set recognizing all members of the genus Leishmania, we determined that all nine human samples (from five humans) contained Leishmania DNA, but domestic animal, rodent, and sand fly samples were all negative. Real time PCR assays using species-specific primer-probe sets could not confirm either L. major or L. tropica as the infectious agent in the human samples. This result is contrary to published information and may indicate several scenarios, including infection by multiple Leishmania species or even novel species. To resolve this question, we are sequencing a fragment of the 16S ribosomal gene (using a primer set that amplifies from all Leishmania species). These data are part of an ongoing study and will be updated accordingly.

(ACMCP Abstract)

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DETECTION OF HISTAMINE IN FISH SOLD IN MARKETS IN LIMA, PERU


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Histamine is produced by decarboxylation of histidine in some spoiled or bacterially contaminated fish due to inappropriate storage. Ingestion of high histamine levels often produces a generalized allergic reaction and is occasionally associated with the consumption of fish like mackerel, tuna, bonito and mahi-mahi. The Food and Drug Administration defines a hazard action level in these fish as 50 ppm.

After a case of histamine-related allergy experienced by a coworker after ingestion of bonito, we evaluated the presence of histamine in fish sold in Lima, Peru. The two wholesale seafood markets that supply all Lima and 15 general public markets were visited during five Mondays between 4 AM and 4 PM in April-May 2006. Fish from three species were sampled: bonito (Sarda chilensis chilensis), mackerel (Scomber japonicus japonicus) and mahi-mahi ( Coryphaena hippurus). Wholesale markets were visited twice each, all other markets were visited once. One whole, uncut fish was bought per seller, and sellers were not told that fish would be tested. Histamine levels between 0-50 ppm were measured by a quantitative ELISA (Veratox®) at Naval Medical Research Center Detachment, Lima, Peru. Dilution methods with the same kit were used to estimate approximated concentrations above this range. We tested 38 fish (17 bonito, 16 mackerel, 5 mahi-mahi), 13 fish (32%) from wholesale seafood markets, including all mahi-mahi. Six fish had histamine levels 1-5 ppm (3 mackerel, 3 bonito) and four had > 5 ppm (3 mackerel, 1 bonito), all from general markets except for one mackerel with 2.8 ppm. Three mackerels bought between 2 and 4 AM had 35, 83 and 86 ppm, respectively. Fish from general markets had histamine levels > 5 ppm more frequently than fish from wholesale markets (44% vs. 8%, p = 0.03). Higher histamine counts correlated with later time of purchase (Spearman’s = 0.37, p = 0.024). A sample of the bonito ingested by the original case showed over 800 ppm of histamine. Food safety is an important concern in Peru, where fishing is an important industry and seafood is a key element of local cuisine. This pilot study highlights the risks associated with seafood related intoxication. Inappropriate freezing procedures in the transport and selling of fish may be allowing production of histamine. Although this preliminary evidence precludes us from making broader conclusions, it clearly emphasizes the need for further research on seafood safety in Peru.

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SURVEILLANCE OF ACUTE RESPIRATORY INFECTION IN CHILDREN FROM DIFFERENT REGIONS OF PERU: COMPARISON OF PERUVIAN NAVY AND MINISTRY OF HEALTH DATA

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Since January 2003, the Peruvian Navy has collected weekly health data from military personnel and their dependants through an electronic surveillance system called Alerta. Acute respiratory infections (ARI) are a significant cause of morbidity. Information on the epidemiology and seasonality of these infections are crucial in planning health care services and preventive measures. This study was undertaken to describe the distribution of ARI cases in the population of children from the Peruvian Navy and compare it with Ministry of Health data. We reviewed all ARI cases in children younger than 5 years old from January 2004 to December 2005 collected by Alerta. Data from the General Direction of Epidemiology (DGE) were obtained from the same population during the same period. The data were grouped in four geographic regions: North coast, Central coast, South coast and rainforest. No data were available from the highlands. Data were described through number of cases, number of cases per health setting and number of cases per health setting per week. Time series correlation between Alerta and DGE and between Alerta regions was performed by means of linear regression analysis. From 2004-2005, Alerta collected 6406 cases. 578 (9%) corresponded to the North coast, 4684 (73%) to the Central coast, 257 (4%) to the South coast and 887 (14%) to the rainforest. The mean number of cases per health setting per week according to the Alerta system was 2.95 for the North coast, 15.71 for the Central coast, 1.89 for the South coast and 3.82 for the rainforest.

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For DGE, the rates were 15.44 for the North coast, 16.40 for the Central coast, 15.61 for the South coast and 9.46 for the rainforest. There was a significant correlation between Alerta and DGE data for the North coast ($R^2 0.322, p=0.0079, r^2 0.07$), for the South coast ($R^2 0.193, p<0.0001, r^2 0.26$) and for the rainforest ($R^2 0.939, p=0.0001, r^2 0.14$). No correlation was found for the Central coast or between Alerta regions. In conclusion, the data on ARF cases collected through the Alerta system showed that each region correlated with the DGE data, except for the Central coast. There was no correlation between different geographic regions according to this system. This analysis validates Alerta data and highlights the need for surveying different populations.

VALIDATION OF THREE DIFFERENT ALGORITHMS FOR OUTBREAK DETECTION IN ACUTE DIARRHEAL DISEASE IN PERU

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Surveillance systems are powerful tools for gathering baseline data on incidence of disease and detection of outbreaks. Automated algorithms allow the rapid use of data generated by surveillance systems. “Alerta” is an electronic surveillance system in the Peruvian Navy that collects health data from multiple sites and transmits them to a central hub for analysis. We evaluated the usefulness of several algorithms to automatically detect outbreaks of acute diarrheal disease (ADD), the most common illness reported. We reviewed the ADD reports from Aug03-Jun05 for 2 settings: the Ancon Navy Base and the more complex Callao Base Hospital (HOBAC). Three algorithms were applied to calculate maximum expected values based on the ten previous weeks. The X-bar chart uses the average of the previous number of cases. The Moving Range chart uses the average of the variations between weeks. The Cumulative Sums chart (CUSUM) adds the differences between frequencies and their expected means. Three standard deviations were used as upper limits for an outbreak signal. Sensitivity, specificity, Positive Predictive Value (PPV) and concordance values were calculated for each algorithm and setting. Data from 96 weeks were evaluated. The Ancon Base reported 3 outbreaks with an incidence of ADD of 8.86 cases/week per 1000 persons. At HOBAC, 8 outbreaks were reported with an incidence of 1.43 cases/week per 1000 persons. In the Ancon base, all the algorithms had 100% sensitivity, while the specificity was 95% for the X-bar, 98% for CUSUM and 99% for the Moving Range. PPV was 43%, 60% and 75% respectively. At HOBAC, all the algorithms had 75% sensitivity and 87% for the X-bar, 92% for CUSUM and 97% for moving range. PPV was 38%, 50% and 75% respectively. Concordance was lower between X-bar and Moving Range chart (94.2% with a $\kappa=0.517$ for the Ancon base and 88.2% with $\kappa=0.524$ for the HOBAC base). In conclusion, all 3 algorithms were useful for the detection of ADD outbreaks. While there were essentially no differences in sensitivity between algorithms, the specificity and PPV were higher for the Moving Range chart. The sensitivity and specificity were lower in a more complex surveillance setting, like HOBAC. High concordance between algorithms was observed.

PREVALENCE OF ANAPLASMA PHAGOCYTOPHILUM AND BORRELIA BURGDORFERI SS IN TWO HIGH RISK HABITATS IN NORTHWESTERN CALIFORNIA

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Landscape level conversion of forest may have contributed to emergence of tick-borne diseases including Lyme borreliosis (LB) and human granulocytic anaplasmosis (GA) in the eastern US and Europe and may be relevant in California as well. Human cases of GA commonly occurred in people who lived adjacent to coast redwood forests, although ticks are rarely recovered from redwood forests. In California, both LB and GA occur in hyperendemic foci with a high degree of variation in prevalence across different landscapes. Both pathogens are transmitted by the same tick vector, *Ixodes pacificus*. In this study we measured tick density and diversity, wild rodent diversity and abundance, and prevalence of *Anaplasma phagocytophilum* from four sites in northwestern California. At each site, twelve 50m transects were evenly distributed between old-growth redwood and oak forests and second-growth forests. Ticks were collected directly from wild-caught rodents two times/year and by flagging every 2 months along each transect and evaluated for *A. phagocytophilum* infection using Taqman PCR. Wild rodents were trapped by placing 10 Sherman live traps on each transect for 3 trap nights. Rodents were bled and analyzed for *B. burgdorferi* and *A. phagocytophilum* infection using both serology and PCR. Preliminarily, 8 species of rodents and six species of hard ticks, including *cosmopolitan I. pacificus and D. variabilis*, as well as 3 rodent-specialist ticks, were captured and analyzed for infection of tick-borne disease. Prevalence of GA in rodents varied across sites from 1-12% and among rodent species from 0-75%. A phagocytophilum PCR-positive test results were obtained for dusky-footed woodrats and tree squirrels, but not in other rodents or in ticks, while *B. burgdorferi* PCR-positive test results were detected in dusky-footed woodrats. Tick and rodent density appeared higher in oak, compared to redwood, communities, but a clear pattern of increased tick density in second-growth forest has not been detected.

RELAPSING MALARIA INFECTION IN AN ADOLESCENT FOLLOWING TRAVEL TO MOZAMBIQUE

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Travelers to Africa presenting with malaria typically are infected with *Plasmodium falciparum*. Infection with *P. ovale* is uncommon and seldom occurs outside of West Africa. A case of relapsing malaria acquired in Mozambique by an adolescent traveler is reported. A 16-year old male presented with a ten-day history of fever to 102.9F, rigors, malaise and diarrhea 60 days after returning from a 2-week trip to Mozambique. He reported full compliance with malaria chemoprophylaxis, mefloquine 250MG weekly beginning 2 weeks pre- and ending 4 weeks post-trip. His primary care physician started atovaquone/proguanil HC100mg BID for 3 days after a positive blood smear. Species identification was not done at that time. After completing a course of atovaquone/proguanil, the patient's symptoms resolved. He then presented 45 days later with a 2-week history of fatigue and one day of fever, chills, nausea and vomiting. He was hospitalized for concern of recurrent *P. falciparum* malaria and given quinine and doxycycline for 7 days. His blood smear was positive for Plasmodium, suspect ovale. The patient was discharged 3 days after admission, afebrile and clinically improved. His specimen was later confirmed ovale species by PCR testing. He was treated with primaquine 26.3 MG daily for 14 days without further recurrence of symptoms. In conclusion, malaria in former travelers may not present until months after the trip. In patients with recurring symptoms, ovale malaria must be considered even in travelers to areas in East Africa such as Mozambique that historically have been thought to have a low prevalence of the ovale parasite. Diagnosis by PCR can be useful in these patients since accurate microscopic species identification is not always possible.

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PREVALENCE OF BORRELLIA BURGDORFERI, BARTONELLA SPP., BABESIA MICROTI, AND ANAPLASMA PHAGOCYTOPHILUM IN IXODES SCAPULARIS TICKS REMOVED FROM HUMANS

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Ixodes scapularis parasitizing Department of Defense (DOD) personnel and their dependents were received by the DOD Human Tick Test Kit Program in 2005 and tested by PCR for Borrelia burgdorferi, Babesia microti, and Anaplasma phagocytophilum. Most of these I. scapularis were acquired in the upper Midwest, mid-Atlantic, and New England regions of the U.S.; a very few were acquired in the southern U.S. Of a total of 389 I. scapularis, 78/389 were infected with B. burgdorferi, 3/389 were infected with A. phagocytophilum, 6/389 were co-infected with B. burgdorferi and A. phagocytophilum, 6/389 were co-infected with B. burgdorferi and B. microti, and one was co-infected with A. phagocytophilum and B. microti. Because of concerns about tick-borne Bartonella infections voiced by patient groups and physicians, and because of evidence of Bartonella sequences amplified from I. scapularis ticks, further PCR of these 2005 ticks is being conducted using primers for the gltA gene of Bartonella spp. and will be presented. Infection rates from the 2006 tick season will also be presented.

JUVENILE TICK SURVIVAL ESTIMATION AND APPLICATION TO A PREDICTIVE MODEL FOR ANAPLASMA PHAGOCYTOPHILUM PERSISTENCE IN NATURE

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Granulocytic anaplasmosis (GA) is an emerging tick-borne disease caused by infection with Anaplasma phagocytophilum and maintained throughout the Holartic in sylvatic cycles involving ixodes spp. ticks. In the western US, the most important bridge vector to humans and domestic animals is Ixodes pacificus, with ridiculous rodent-specialist ticks playing a role in enzootic cycles. To some extent, infection over-winters in ticks during tick hibernation; in California, there is no obvious reservoir host the host with the most prolonged infection, the dusky footed woodrat, Neotoma fuscipes, typically experiences infection for a few weeks to as long as 6 months. A vector-SIRS model of transmission of A. phagocytophilum among apparently poorly competent hosts such as woodrats and several tick species was constructed to explore critical parameters driving disease dynamics, emergence, and persistence. Sensitivity analysis indicated that the single most important model parameters for predicting disease persistence was survival probabilities of juvenile ticks. Juvenile I. pacificus survival was experimentally determined in situ, by placing larvae and nymphs in tubes in leaf litter at several field sites that differed in microhabitat features (soil temperature and humidity, substrate), that would modify tick survival. Using these survival estimates with parameter values derived from an A. phagocytophilum-enzootic community in northern California, the model yielded critical survival thresholds for disease persistence, corresponding to experimental results where microhabitats had mild temperatures and high humidity.

DENGUE VIRUS TYPE 3 IN CUBA: EVOLUTION FROM A SMALL OUTBREAK IN 2000 TO A MAJOR EPIDEMIC IN 2001

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After an absence of 17 years, DENV-3 re-appeared in Latin America in 1994. Cuba was first affected in September 2000 when a small outbreak occurred in Havana City. The infection was brought under control within six weeks using enhanced mosquito eradication measures. In June 2001, dengue transmission was again detected, this time the virus spread rapidly across the city causing a major epidemic. To understand the phylogenetic relationships of the viruses isolated from these outbreaks, the E gene sequence of three Cuban isolates and the first DENV-3 strain isolated in Nicaragua, 1994 were determined. Maximum Likelihood phylogenetic analysis incorporating global DENV-3 sequences showed that the Cuban isolates are closely related to strains belonging to genotype III and formed a distinct cluster with recent Latin American strains that have evolved in the Caribbean region. Analysis of Cuban isolates obtained in consecutive outbreaks revealed several nucleotide changes, some of them associated with non-conservative amino acid substitutions. These data are therefore consistent with the idea that a second introduction of the virus occurred in 2001, rather than in situ evolution. The functional significance of amino acids changes that were observed remains to be determined. Moreover, it is noteworthy the amino acid change observed among isolates obtained during the same outbreak from patients with different disease severity as well as from different biological sample (serum or spleen).

DENGUE HEMORRHAGIC FEVER CAUSED BY SEQUENTIAL DENGUE 1 - 3 INFECTIONS AT A LONG INTERVAL: HAVANA EPIDEMIC, 2001-2002

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A DENV-3 epidemic occurred in Cuba in 2001-2002 which included cases of dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). Here we report neutralizing antibody studies on sera from 54 of 78 DHF/DSS patients that provide evidence of infections occurring in the sequence DENV-1 followed by DENV-3. No sera evidenced infection in the sequence DENV-2 followed by DENV-3. Some sera showed a pattern of infection in the sequence DENV-1 followed by 2 then 3, however definitive categorization of a tertiary infection was not possible because of broadly reactive antibodies which could have been raised by infections in the sequence DENV-1 then DENV-3. Dengue Hemorrhagic Fever has been associated with secondary infection in individuals who experienced a primary dengue infection 3-5 years earlier. In this manuscript two important observations are reported: a secondary dengue infection is demonstrated as an important risk factor for severe disease occurring 24 years after a primary dengue infection and b. The infection sequence, dengue 1 followed by dengue 3 was associated with severe disease. There was no evidence that dengue 2 followed by dengue 3 infections resulted DHF/DSS, although infections in this sequence leading to milder illnesses were observed. These two observations are new and of importance to understanding the pathogenesis of this disease and in vaccine safety issues.
specific immunoglobulin M, A and E in primary and secondary dengue infection from Cuban adults and Salvadorian children

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Dengue IgM antibody detection in a single acute-phase serum by ELISA has become one of the most important and useful methods for the diagnosis of this disease. Currently, this system has become an invaluable tool for the surveillance of Dengue Fever and Dengue Hemorrhagic Fever. The usefulness of other serological markers such as IgA and IgE in serum has been less studied. One hundred twenty seven serum samples from adult patients of the Cuban dengue 3 epidemic of 2001-02 and seventy one serum samples from children patients collected during the dengue 4 epidemic of El Salvador (2002) with clinical picture of dengue fever or dengue hemorrhagic fever and with primary or secondary infection were studied. All samples were tested by capture ELISA in order to detect dengue IgM, IgA and IgE antibodies. Significant differences were observed in the IgM, IgA and IgE response in each studied group. Higher OD ratios for IgA and IgE antibodies in secondary dengue cases than primary cases were found. The usefulness of serotype specific IgM antibody detection is also analyzed and discussed. The role of these immunoglobulins in terms of protection, recovery of infection and immunopathogenesis is a priority in future dengue investigations. Cross-reactivity of IgM among dengue serotypes both in primary and secondary cases should be carefully studied.

improved dengue plaque virus formation on BHK21 and LLCMK2 cells: evaluation of some factors

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Neutralizing antibodies play a key role in the prevention of dengue infection. It is important that dengue virus plaque titration and plaque reduction neutralization tests (PRNT) be highly reproducible using standardized methods. To evaluate factors that have influence in the PRNT for dengue viruses, neutralizing antibodies were determined in 24 serum samples and 12 blood samples collected on filter paper, obtained through Cuban national dengue surveillance. The influence of pH in the overlay medium, percentage of ambient CO2, the use of two different cell lines and of rapid centrifugation on dengue plaque formation were evaluated. The efficiency of the plaqueing system was optimal when overlay medium was buffered to pH 7.5. The rapid centrifugation of virus on confluent cells increased the virus titers. Higher virus titers were obtained on BHK21 rather than LLCMK2, cells when viruses were added to cell suspension. Under optimal conditions, PRNT was highly reproducible and is recommended for seroepidemiological and vaccine studies using either BHK21 or LLCMK2, cells. This communication also highlights the infection of LLCMK2, cell suspensions for measuring neutralizing antibodies.

antibodies dependent cell cytotoxicity in dengue infection

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The role of humoral immunity and neutralizing antibodies in dengue virus disease has been studied. In addition to direct interference with viral entry achieved by neutralizing antibodies, antibodies in vivo mediate another important function less explored in dengue infection: the antibodies depending cell cytotoxicity (AECC). Like CTL activity, ADCC could eliminate infected cells and thereby reduce viral burden. Unfortunately, only scarce reports on ADCC during a dengue infection are currently available, and they do not clearly define ADCC role in the prevention or progression to DHF/DSS. The exceptional epidemiological circumstances in Cuba allow us to maintain a homogeneous sample with a similar history of natural infections. In this study we explore the ability of human anti-dengue antibodies to mediate ADCC using acute or convalescent serum samples from individuals who suffered secondary infection to dengue 2 virus in the epidemic of Santiago de Cuba in 1997 with different clinical pictures. All these individuals had been primarily infected by the dengue 1 virus 20 years before. According to the clinical picture, ADCC activity was detected at fifth day after clinical onset, in sera from DHF/DSS but not in sera from DF patients. However, one year after illness, ADCC activity was observed in all cases. We also measured the activation of PBMC mediated by antibodies detection by IFNγ. In order to do so, we used serial serum samples from these patients collected every two days after fever onset. In DF samples taken between the first and third day, a higher number of IFNγ positive cells was detected, and a decrease on the fifth day. However, for DHF cases there was no detection of IFNγ positive cells in the first samples after clinical onset, but there was an increase towards the fifth day. The analysis of specific anti-dengue IgG subclasses in the acute serum samples studied showed an IgG2 major contribution to the IFNγ production. Our results suggest that the ADCC antibodies present during the earlier stages acute infection could play a role in determining the viral spreading, and consequently avoid the progress to the severe disease. Then, ADCC could be implicated in dengue prevention.

A dengue virus vaccine based on alphavirus replicons induces protective immune responses in cynomolgus macaques

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A candidate vaccine (D1ME-VPF) expressing dengue virus type 1 pre-membrane (prM) and envelope (E) proteins from a Venezuelan equine encephalitis virus (VEE) replicon system was previously shown to elicit both anti-dengue-1 neutralizing antibodies and dengue-1 specific CD4+ and CD8+ T cells in a murine model. In this study, three vaccination regimens (D1ME DNA vaccine, D1ME-VPF, and a heterologous prime boost with D1ME DNA prime and D1ME-VRP boost) were compared for immunogenicity and protection against dengue-1 virus challenge in a non-human primate model. Groups of 3 or 4 cynomolgus macaques were immunized with three doses of D1ME DNA vaccine (DD1), D1ME-VRP (VV1), or with two doses of DNA priming and a third booster dose of D1ME-VRP (DDV). A control group of animals was inoculated with PBS. Virus neutralizing antibody was measured by plaque reduction neutralization test (PRNT) and 50% neutralization titers (PRNT-50) were determined by probit analysis. T cell responses were measured by γIFN ELISPOT. Measured 4 weeks after final immunization, the DDV group produced the highest virus neutralizing antibody titers (PRNT-
Differential Enhancement of Dengue Immune Complex Infectivity Mediated by Signaling-Competent and Signaling-Incompetent Human FC γ RIA (CD64) or FC γ RIIA (CD32)

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FCγ receptor (FcγR)-mediated entry of infectious dengue immune complexes into monocyte/macrophages is hypothesized to be a key event in the pathogenesis of complicated dengue fever. FcγRIIA and FcγRIIB, Fc receptors that predominate on the surface of such dengue-permissive cells, have previously been shown to facilitate antibody-mediated dengue enhancement in human macrophage-like cells using surrogate plaque assay models to measure virus replication since dengue virus does not form plaques in such cells. We have examined the relative efficiency with which each of these receptors individually enhances dengue immune complex infectivity and have inquired whether Fc receptor signal transduction plays a role. Our strategy is to answer these fundamental questions surrounding the immune enhancement phenomenon involved in expression of innate and adaptive immune mechanisms. In dengue-permissive COS cells in which dengue virus immune enhancement was directly measured by conventional plaque assay. We found that both receptors mediated enhanced dengue immune complex infectivity, but that FcγRIIA appeared to do so far more effectively. Abrogation of signaling competency significantly diminished the capacity of FcγRIIA transfectants to phagocytose opsonized large particles and to enhance dengue immune complex infectivity. Abrogation of FcRIIA signaling competency was also associated with equally impaired phagocytosis, but had no discernible effect on dengue immune complex infectivity. These findings point to fundamental differences between FcγRIIA and FcγRIIB with respect to their immune-enhancing capabilities and suggest that different mechanisms of dengue immune complex internalization may operate between these FcγR.

Gene Polymorphisms of Immunoregulatory Cytokines in Dengue Virus Infection

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Dengue virus infection has emerged as one of the most important arthropod-borne diseases. Some infected individuals progress to the severe, life-threatening form of the disease, dengue hemorrhagic fever. Host genetic factors may be relevant and predispose some individuals to the severe dengue disease. The unique history of Dengue outbreaks in Cuba is extremely advantageous for genetic studies of dengue disease resistance or susceptibility. Little is known about predictive value of cytokine genotype for the development of clinical outcome of dengue infection. The TNF-α, -869G/A, -592G/A, and -308G/A gene single nucleotide polymorphisms (SNP) were studied in individuals who suffer from different clinical pictures or subclinical dengue virus infection by polymere chain reaction-sequence specific primer (PCR-SSP). Significant association of the tumor necrosis factor-α (-308) GG genotype was found when comparing asymptomatic and dengue hemorrhagic fever cases. No associations of interleukin-10 polymorphisms with any studied groups were detected. We failed to observe significant differences in cytokine genotype distribution between dengue fever and dengue hemorrhagic fever patients.

Differential Virus-Specific T-Cell Response in Dengue Virus Immune Cuban Individuals

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Dengue virus (DV) infections play an increasing role in the world. The rapid activation of serotype cross-reactive dengue memory T cells that release shock-provoking inflammatory mediators has been suggested to explain some aspects of the severe clinical syndromes. How T cells contribute to this process, however, is incompletely defined. We take advantage of the unique history of dengue in Cuba, where the population has been exposed to the identical previous dengue infection in each outbreak to study how the rapid activation of serotype cross-reactive memory T cells that release shock-provoking inflammatory mediators contribute to the development of the severe clinical syndromes by mean of quantifying...
CIRCULATING DENGUE VIRUS SEROTYPES SINCE THEY ENTERED PERU IN 1990 UNTIL 2006

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This study was undertaken to identify dengue virus serotypes circulating in Peru since 1990 up to 2006. The information available regarding dengue fever virus isolates in the Virology Laboratory of the Peruvian National Institutes of Health (PINH) was reviewed, comprising the time period since 1990 to the first quarter of 2006. Viral isolation at the PINH is performed using VERO and C6-36 cell cultures, as well as inoculating the material in brain tissue of lactating mice. Samples are collected from laboratories located in different endemic regions for dengue fever in Peru. Viral identification is performed using direct immunofluorescence with monoclonal antibodies. When necessary, PCR or genotyping techniques are used. Dengue virus serotype identification corresponded to dengue (DEN) 1 between 1990 to 1995; 1996: DEN2; 1997: DEN1; 1998: DEN2; 1999: DEN1; DEN2: 2000: DEN1; 2001: DEN1; 2002: DEN2; 2003: DEN2; 2004: DEN3; 2005: DEN1, DEN3 (Lima); 2006: DEN 3. Circulating DEN2 in 1996 corresponded to DEN2 American genotype. Serotype 3 entering Lima corresponded to genotype 3. Dengue virus entered Peru by the Amazon Region and spread through all the Northern Coast, from Tumbes to Lima, as well as in the Amazon Region. In conclusion, dengue virus type 1 entered Peru in 1990; and since then, the 4 serotypes of dengue fever virus have been circulating, and there is a constant risk for the occurrence or re-emergence of hemorrhagic dengue fever in endemic areas in Peru.

STUDYING CROSS NEUTRALIZATION OF DENGUE VIRUSES WITH A PANEL OF DENGUE IMMUNE-SERA FROM TRAVELERS

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A central tenet in dengue immunology is that following natural infection with a particular serotype, one develops long-term protective immunity to the infecting serotype but not to the other serotypes. Recent studies indicate, however, that genetic differences between viruses belonging to the same serotype can influence the extent of neutralization. Studies by Kochel et al. have demonstrated that people exposed to primary dengue serotype 1 (DENV1) developed cross-reactive immune responses that neutralized the American but not the Asian DEN2V. We are performing a comprehensive analysis of DENV3 neutralization, asking whether homotypic immunity that develops after a primary DENV3 infection neutralizes all DENV3 strains to the same extent irrespective of genetic differences within this serotype, and whether homotypic immunity that develops after a primary DENV1 or DENV2 infection cross-neutralizes some strains of DENV3 better than others. A major obstacle to studying cross-neutralization of dengue viruses is the scarcity of monotypic dengue immune sera. We have attempted to overcome this problem by screening American travelers who developed dengue-like symptoms during a visit to a dengue endemic country. Unlike people living permanently in dengue endemic areas who have repeated dengue infections, traveler’s sera are likely to remain monotypic for many years after the infection. The travel histories can be used to determine when and where the person was exposed to the virus. We obtained serum and PBMC from 33 people who were likely to have been infected with dengue virus. 16 of the 33 subjects had high levels of neutralizing antibody to at least one serotype, whereas the remaining subjects were dengue naive or had very low levels of neutralizing antibodies. Of the 16 positive subjects, seven had monotypic responses and the remaining nine had responses indicative of secondary infections. Experiments are currently underway with these sera to measure neutralization of 7 strains of DENV3 representing the genetic diversity within this serotype.

IMPROVEMENT IN HOSPITAL INDICATORS AFTER CHANGES IN DENGUE CASE MANAGEMENT IN THE NATIONAL PEDIATRIC HOSPITAL IN NICARAGUA

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Dengue is a major problem in Nicaragua, as in many tropical and subtropical countries worldwide. Improving dengue case management and quality of care are a significant priority. Via a collaboration with Thai colleagues at the Queen Sirikit National Institute of Child Health, changes in management of suspected dengue cases were introduced in the National Pediatric Reference Hospital in Managua, Nicaragua. These modifications consisted of oral liquids rather than IV fluids upon admission, continuous monitoring of clinical and laboratory signs, introduction of a microhematocrit centrifuge on the ward for frequent surveillance to detect increased vascular permeability, use of IV fluids principally during the critical phase and for shorter periods, and introduction of colloids in management of shock. To assess the impact of these measures, two periods were compared, representing the 2003 and 2005 dengue seasons, before and after the implementation of these new practices. Apart from these specific changes, there were no other differences in case definition, management, or disease severity between the two periods. For instance, 29% of hospitalized dengue patients were classified as dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) in 2003 versus 26% in 2005. A number of outcomes were evaluated, including nosocomial infections, intensive care, duration of hospitalization, number of patients receiving IV fluid, and day of initiation of IV fluid. In 2005, 206 cases of laboratory-confirmed dengue cases were beds for patients of age who presented at the hospital ≤ 4 days after onset of symptoms were included in the study, versus 46 in 2005. Some of the most important outcomes were a dramatic reduction in nosocomial infections, from 25 in 2003 to 0 in 2005 (p=0.04) and in admissions to the intensive care unit, from 8 in 2003 to 0 in 2005 (p=0.44). Other significant findings included reduction in (i) the days of IV fluid administration (p=0.001), (ii) the number of patients receiving IV fluids (p<0.0001), and (iii) duration of hospitalization (p=0.003) Overall, this study demonstrates concrete gains in dengue patient care and case management.

IDENTIFICATION OF CONTINUOUS B-CELL EPITOPES IN THE ENVELOPE GLYCOPROTEIN OF DENGUE VIRUS TYPE 3

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Dengue virus infection is a growing global public health concern in tropical and subtropical regions of the world. The virus is a single-stranded RNA virus that belongs to the Flaviviridae family with 4 antigenically distinct serotypes (DENV-1 to DENV-4). There is no antiviral therapy available and...
development of a dengue vaccine has proved to be elusive due to the requirement of the vaccine to elicit protection against all four serotypes simultaneously. One possible strategy to avoid pathology associated with a dengue vaccine would be to construct a chimeric vaccine composed of selected critical epitopes of the four serotypes. The majority of the epitopes involved in dengue neutralization are on the envelope (E) glycoprotein, which is the major surface protein of the viral particles. The aim of the present investigation is to identify B cell epitopes in the E-glycoprotein elicited by natural dengue virus type 3 infections. For mapping immunodominant epitopes, ninety five peptides (each with 15mers, overlap of 10) were synthesized (Synpep, California-USA), covering the 490 amino acids (aa) of the E-protein sequence deduced from the genome of a Dengue 3 isolate from Brazil. These peptides were tested by ELISA against a pool of positive and negative dengue patient sera collected during the convalescent phase of dengue 3 infection, as determined by PCR. The results showed that the human sera reacted with eleven of the 15-mer peptides, distributed in 5 regions at amino acid positions 51-65, 71-90, 131-170, 196-210 and 246-260 and all of these, except the peptides 196-210 and 246-260 are hydrophilic according to Kyte and Doolittle hydroplicity plots suggesting that these regions are exposed at the surface of the E protein. In conclusion, our study identified several immunodominant IgG-specific epitopes on the envelope of DENV-3. The peptides described here in conjunction with other well-documented epitopes are potentially relevant for the development of diagnostic reagents and vaccine for the dengue virus.

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IDENTIFICATION OF DENGUE VIRUS IN PATIENTS WITH INESPECIFIC FEBRILE ILLNESS

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In endemic areas for dengue infection, the unnoticed transmission is a common situation that could provoke an underestimation of the actual incidence, especially in the absence of epidemic outbreaks. The purpose of this descriptive study was to explore the association between dengue infection, their serotype and clinical diagnosis in a hyperendemic area for dengue transmission. A total of 137 consecutive patients attended at the public health services in the state of Colima, Mexico, with the clinical diagnosis of inespecific fever were included in the study. Clinical and demographic data were recorded. A venous blood sample was obtained in each patient in order to look for the presence of dengue virus and its serotype by means of the transcriptase reverse- polymerase chain reaction (RT-PCR) with specific primers for the four dengue serotypes. A total of 18 sera resulted positive to dengue infection (13.1%), 16 of them corresponding to DEN-3 type and 2 of DEN-1 serotype. The analysis with Poisson regression did not show association between dengue infection with sex, age group, type of community nor with clinical picture (only 2 patients had the clinical diagnosis of dengue). The result confirms the recent re-introduction of DEN-3 serotype to Mexico. On the other hand, the findings support our previous findings that dengue transmission occurs in endemic communities in a continuous fashion, even in the absence of epidemics and that clinical diagnosis usually is not enough specific or sensitive to be considered as a reliable tool for epidemiologic surveillance.

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GENETIC CHARACTERIZATION OF DENGUE VIRUS SEROTYPES CIRCULATING IN OAXACA, MEXICO

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Dengue fever (DF) and dengue hemorrhagic fever/shock syndrome (DHF/DDS) are mosquito-borne infectious diseases that have become major international health concerns. DF and DHF/DDS occur in tropical and subtropical regions around the world, predominantly in urban and sub-urban areas. There are four dengue serotypes, which are transmitted to humans principally through the bites of Aedes aegypti. Sequential infection with different serotypes could be increases the risk of DHF, and this may be associated with the potential virulence of the strains of DEN virus. A number of genetic markers have been proposed to condition these increased virulence, and recombination event could result in the generation of new, more virulent dengue viral genotypes. In this work, we studied the circulation of serotypes of DEN virus for serotification of 6 isolates obtained of patients serum of the state of Oaxaca, Mexico. The maximum cytopatic effect (CPE) produced by virus DEN infection was obtained after three passage about 30 days in the mosquito cell lines C6/36. The results of RT-PCR indicates that there are three DEN virus serotype 2 (DENV-2) and three DENV-3. This study indicates that several serotypes are circulating in Oaxaca increasing the risk of DHF. We made the sequence of protein E, and a portion of the C and prM genes. Phylogenetic analysis suggested that the isolate of DEN serotype 2 was American/Asian genotype, which has DHF potential. The sequence analysis of NS5 compare with the genotype elucidated by the aminoacid sequence of protein E, suggested some genetic markers in NS5 to genotype DEN virus serotype 2.

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CHLOROQUINE REDUCES DENGUE VIRUS REPLICATION IN VERO CELLS BUT NOT IN C6/36 CELLS, AND PARTIALLY PROTECTS MICE AGAINST VIRAL CHALLENGE

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Dengue represents the most important arboviral disease of humans. The only available way to control dengue is vector control since there is neither a vaccine to protect against this infection nor an antiviral that interferes with virus replication. To investigate whether chloroquine would interfere with dengue virus replication, Vero and C6/36 confluent cell monolayers were infected with dengue-2 virus (DEN2V) at a multiplicity of infection of 0.1. Viral replication inhibition assays were performed on Vero and C6/36-infected cells treated with chloroquine (50μg/mL) added either at 12- or 24-hour intervals after adsorption. Infected cell supernatants were collected after 0, 6, 12, 24, 48, 72, 96, 120, 144, and 168 hours after viral infection. Total RNA was extracted from these supernatants, and viral replication was assessed by real-time PCR. Reverse-transcription real-time PCR results showed that, compared to control cells, there was a statistically significant decrease in viral replication in chloroquine-treated Vero cells. The inhibition of viral replication was more striking on those cells treated with chloroquine at shorter time intervals. However, in C6/36 cells chloroquine induced a statistically significant increase in viral replication after 12 hours of infection when compared to control cells, probably because DEN2V uses a different strategy of penetration or replication in these cells. In order to investigate the potential of chloroquine on DEN2V infection, groups of 4-week-old Swiss mice were challenged with an intracerebral injection of a wild strain of the DEN2V, and treated with chloroquine by the intraperitoneal route. The chloroquine was administrated at 24-hour intervals during 7 days, and the animals observed during 21 days. The survival rates of dengue-2-infected mice treated with chloroquine were higher than in the untreated mouse group.
(33% and 0%, respectively). This work shows that chloroquine interferes with dengue-2 replication in vitro and in vivo, and might represent an alternative to treatment of dengue infections in the near future.

CONFIRMATION OF AN OUTBREAK OF SELVATIC YELLOW FEVER IN A NATIVE COMMUNITY IN THE PERUVIAN AMAZONIAN JUNGLE

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Yellow fever (YF) is still a worldwide important disease for public health, even tough an effective vaccine is available. In South America, Peru is one of the countries where YF is most prevalent. Endemic areas are northern and central parts of Peruvian Amazonian jungle. In December 2005, there was an outbreak of a condition characterized by fever, jaundice, and hemorrhage in a native community (the Awajun tribe) comprising 435 inhabitants in the Amazonian region, in a place very close to the Ecuadorian border, 700 meters above sea level. 106 febrile patients required medical attention, 33 presented with jaundice, 20 had hemorrhage, and 12 patients died. This study was undertaken to determine the causal agent of the condition characterized by fever, jaundice, and hemorrhage in the Awajun native community. We obtained serum and liver tissue samples from the affected patients. EUSA tests were performed for detecting IgM antibodies against yellow fever. Serum aliquots were also inoculated in cells for culture and in suckling mice. RT-PCR, as well as nucleotide sequencing was also performed in the serum samples and isolation, respectively. According to serology tests, viral isolation and RT-PCR yellow fever virus was the causative agent for the outbreak. Gene sequencing showed that this virus had 99% and 88% coincidence with other yellow fever viral sequences reported in Gene Bank from South America and Caribbean region, respectively. This outbreak had some particular features; its elevated attack rate; the occurrence of affected children less than 5 years old; the native population has never been immunized against yellow fever; there were no reports of dead monkeys; and no epizootics were reported in the affected area; and it was suspected that transmission occurred inside the village or in its peripheral area. Haemagogus, Sabethes and other mosquito vectors were found.

EXPERIMENTAL EVIDENCE THAT RNA RECOMBINATION OCCURS IN JAPANESE ENCEPHALITIS VIRUS

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Japanese encephalitis (JE) virus, a major cause of acute viral encephalitis in human, is a member of the genus Flavivirus belonging to the family Flaviviridae. Due to the instability of genomic RNA, mutations accumulated during virus replication is known to be a factor contributing to the viral evolution. In past years, RNA recombination has also been postulated to be another factor to cause genomic variation of JE virus. The first evidence, based on phylogenetic analysis on the E protein, demonstrating RNA recombination of JE virus was reported in 2003; showing at least two strains of JE virus isolated from Korea may be a recombinant form of strains originated from Japan and Korea, respectively. In the meantime, one strain from Thailand was shown possibly formed by strains originated from China and Thailand, respectively. Recently, we have experimentally demonstrated the occurrence of RNA recombination in JE virus by using two local strains isolated from Taiwan. The recombinant progeny virus has actually formed, based on the results of restriction fragment length polymorphism (RFLP), in BHK-21 cells that has been coinfectected by two strains of the parent virus. In addition, two types of subgenomic viral RNA, one contains the 5'-UTR with a short RNA fragment at the 3'-end and the other has the 3'-UTR and the same RNA fragment at the 5'-end, were obtained via in vitro RNA-dependent RNA polymerase (RdRp) assay, from which a newly formed RNA containing both 5'-UTR and 3'-UTR has been identified. Further demonstrated that RNA recombination really occurs during the replication of two co-existing strains of JE virus.

CD4+ T CELLS MEDIATE WEST NILE VIRUS CLEARANCE FROM THE CENTRAL NERVOUS SYSTEM DURING PRIMARY INFECTION

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West Nile virus (WNV) is a single-stranded positive sense RNA virus that is an important human and veterinary pathogen. Although studies have shown that innate and adaptive immune responses are important in controlling WNV infection, to date, the role of CD4+ T lymphocytes and T-dependent helper responses in modulating infection is poorly understood. In this study, using a mouse model, we examined the function of CD4+ T cells in coordinating a protective immune response against WNV. A genetic or acquired deficiency of CD4+ T cells resulted in a protracted WNV infection in the central nervous system (CNS) that culminated in uniform lethality by 50 days after infection. Mice surviving past day ten after infection had high WNV titers in the CNS compared to wild type mice, even 40 days following infection. Immunohistochemistry of brain tissue samples showed persistent WNV antigen staining in the brains of mice lacking functional CD4+ T cells at twenty days post-infection. Interestingly, the absence of CD4+ T cell help did not affect clearance of WNV in the spleen, suggesting a role for CD4-independent responses in clearing virus in the periphery. WNV-specific IgM levels were similar to wild type mice in CD4-deficient mice early during infection, but dropped up to 20 fold at day 15 post-infection whereas IgG levels in CD4-deficient mice were 2-3 log , lower than in wild-type mice throughout the course of infection. Despite this, T-independent antibody responses were sufficient to neutralize WNV in the blood. WNV-specific CD8+ T cell activation and trafficking to the CNS were unaffected by the absence of CD4+ T cells at day 9, but were markedly compromised at day 15. Based on these results, we suggest that CD4+ T cells protect against WNV infection primarily by sustaining WNV-specific CD8+ T cell responses in the CNS.

CONSTRUCTION AND SELECTION OF HUMAN MONOCLONAL FAB ANTIBODIES TO WEST NILE VIRUS USING A PHAGE DISPLAY COMBINATORIAL LIBRARY

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Passive immunity using immunoglobulin has shown efficacy in treating some patients with West Nile virus infection. This makes the development of humanized anti-WNV antibodies significant. The goal of this study was to construct a Fab antibody phage display library of WNV, and to identify and select clones with neutralizing activities. Total RNA was extracted from PBLC of two immune individuals. FF-PCR was used to amplify the heavy chain Fd and light chains. The amplified genes were sequentially cloned into the recombinant antibody expression vector pComb3-H. After transfecting E.coli XL1-blue, a fab phage library was packaged with helper phage VCS-M13. Five rounds of panning were carried out with WNV E protein domain III and EUSA was used to select binding antibodies. Antigen binding specificity, CDR sequence of VH and VL, and neutralizing activity against WNV were analyzed in vitro. Fab antibody library was constructed with a capability of 7 x10^7 clones/ml. Eight Fab monoclonal antibodies were obtained, which recognized linear F protein domain III. One of these, Fab1, exhibited significant neutralizing activity, and completely blocked 100 pfu WNV from infecting Vero cells, at a concentration 160 µg/ml. The other 2, Fab13 and Fab25, showed weaker neutralizing activity, and incompletely blocked 100 pfu WNV infection at
concentrations of 320 μg/ml and 160 μg/ml, respectively. In conclusion, Fab antibodies may be valuable for immunoprphylaxis or treatment of WNV infection.

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MOSQUITO SALIVARY GLAND GENE EXPRESSION DURING LONG-TERM CYTOPATHOLOGICAL WEST NILE VIRUS INFECTION

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Persistent infection with West Nile virus (WNV) in the vector Culex pipiens quinquefasciatus is associated with salivary gland cell death and a reduction in virus transmission over time. Ultrastructural analyses of mosquito salivary glands infected with WNV suggest that apoptosis and extreme cellular degeneration and vacuolization occur during late stages of WNV infection. The aim of the present study was to explore the molecular basis of cytopathology in mosquito salivary glands during WNV infection. We designed oligonucleotide microarrays to test the hypothesis that genes involved in the physiology of the salivary glands, immunity, cell death, and stress response would be differentially transcribed in WNV-infected mosquito salivary gland cells as compared to uninfected, blood fed control mosquitoes. Expressed sequence tags from cDNA libraries of bacteria-inoculated mosquitoes and blood fed midguts were used to generate 60-mer oligonucleotides for spotted microarray slides. Three mosquito infections were performed, from which 100 mosquito salivary glands were dissected on days 14 and 21 post-infection for microarray analysis. Salivary gland tissues were classically expressed in WNV-infected mosquitoes compared to uninfected controls. These differences are discussed in the context of WNV-induced cytopathology observed by transmission electron microscopy. This study represents the first tissue-specific examination of gene expression during long-term flavivirus replication in a Culex mosquito vector and provides insight into transcriptional changes that accompany long-term mosquito infection.

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EPIZOOTIOLOGY OF WEST NILE VIRUS IN THE CENTRAL RED RIVER VALLEY OF NORTH DAKOTA AND MINNESOTA, USA 2002 - 2006

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The establishment and early history of West Nile virus (WNV) within the central Red River Valley of eastern North Dakota and northwestern Minnesota was chronicled from 2002 to 2006. Host-seeking mosquitoes were collected using Mosquito Magnet® traps, sorted by species and tested for WNV using reverse transcriptase polymerase chain reaction assays. Culex tarsalis was identified as the main WNV vector in the region. Passeine birds were collected and tested for anti-WNV antibodies using epoite-blocking enzyme linked immunosorbent assays. Environmental conditions from 2002 to 2005 produced a natural “field experiment” which demonstrated the differing magnitudes by which environmental temperature and host immunity affected local WNV activity. Despite warm temperatures and high vector abundance, WNV activity was low during its introductory year (≈2002). The next year (2003) was an “epidemic year” for WNV, as indicated by the high number of human cases statewide and high infection rates in the local vector population. Passeine immunity was low, which probably contributed to the epidemic. In 2004, unusually cool weather prolonged vector larval development, adult emergence and arboviral extrinsic incubation period. As a result, WNV activity during 2004 was low and WNV had insufficient time to undergo extensive amplification cycles similar to the situation that occurred during the introductory year of 2002. However, the epidemic conditions of 2003 had produced a high level of immunity in the local bird population in 2004. This immunity carried over into 2005. In 2005, environmental temperature, length of transmission season, and vector abundance were all nearly identical to those of the epidemic year of 2003. Yet the intensity of WNV activity during 2005 was considerably less than that of 2003. The big difference between 2003 and 2005 was the level of passerine immunity. The high prevalence of immunity within passerines during 2005 may have contributed to preventing another epidemic, but it did not totally eliminate WNV activity. Results for the 2006 transmission season will be presented.

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SEARCHING FOR A SMALL MOLECULE INDICATOR OF O. VOLVULUS INFECTION

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An essential element currently lacking in river blindness eradication programs is the means of determining the presence of patent infection. The ability to evaluate this is essential for defining areas of need, monitoring progress of drug administration, verifying absence of infection transmission within an area and assessing reoccurrence within a cleared area. A field test kit allowing aid workers to determine which persons in a population are capable of transmitting Onchocerca volvulus and thus require further ivermectin treatment, and which persons do not will greatly aid river blindness eradication programs. As an approach to this goal, we believe a unique metabolic marker exists in the blood of persons patently infected with O. volvulus that could be used to monitor their infection status. An examination of the behavior and lifecycle of O. volvulus strongly suggests communication via pheromones for a number of behaviors. A key aspect of a pheromone is that it is inherently required to be species specific. Closely related species will use chemically distinct pheromones to avoid attracting inappropriate mates. The core idea is to detect the presence of adult parasites by utilizing the very method they use to attract each other. Rather than limiting ourselves to just a putative pheromone, we are conducting a metabolic profile of plasma samples from infected and non-infected individuals as well as from adult worm extracts obtained from nodules. The aim is to identify and isolate any molecule unique to river blindness. We will present the results of our LC-MS metabolic profiles of adult parasite extracts and methanol extracted plasma from infected and non-infected individuals of the Northwest Province of Cameroon.

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FIELD OBSERVATIONS THAT CHALLENGE THE CURRENT DOGMA CONCERNING THE PATHOGENESIS OF LYMPHATIC FILARIASIS

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A mass drug administration program against lymphatic filariasis has now been in place in Tanzania for the past six years. The experience gained from the field-based observations made in this public health initiative has revealed a number of new questions about the biology of filariasis and also encourages a reassessment of some of the basic beliefs that such mass drug programs are based upon. The Tanzanian National LF Program has treated more than 12 million people along the coast, and area long known for a high prevalence of the disease. The Program has
studied the acceptance of the MDA by the population, the effects of the two drugs (annual ivermectin and albendazole) on the ICT antigenemia and circulating parasite loads, and on the clinical presentation by those affected with elephantiasis and associated disease. The need to show a positive effect on individual’s already affected by the disease has been an important element in the Tanzanian Program and has driven the form of advocacy needed for a successful program; this has not been a major directive in Global Program before. It has been seen that many individuals that have taken the drug annually, as is prescribed by the Global Program, remain infected, the possible reasons for this will be discussed. Likewise, the correlation between ICT positivity and circulating microfilaraemia has been found to be variable in hyper-endemic areas of Tanzania, suggesting that there may be a need for different antigen based tests. An unexpected finding has been the very significant improvement patients suffering from lymphatic filariasis have enjoyed and this is believed to be due an improvement in their ability to resist and combat secondary infections. The field remains a vital “laboratory” for gaining a basic understanding of the disease and its parasitology, and the observations made in the Tanzanian Program lay a strong basis for new research efforts that are likely to enhance the overall efforts to eradicate this disease. This presentation will discuss the type of research that is needed to address these new findings.

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EFFECTS OF ANTI-RICKETTSIA DRUGS ON THE MICROFILARIA SURVIVAL OF DIROFILARIA IMMITIS

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Canine and feline heartworm diseases (Dirofilariais), caused by a filarial nematode, *Dirofilaria immitis*, are transmitted by mosquitoes. Human accidentally infected with *D. immitis* have been reported. Human pulmonary dirofilaria develops when the parasites die, embolize, travel to the lungs, and develop nodule in small branch of the pulmonary arteries. Anti-rickettsia drugs have bactericidal activity against the endosymbiont Wolbachia, required for fertility and survival of the filarial nematodes. Our study showed that doxycycline was the most effective compound. After 24 hours in culture, only doxycycline affected microfilariae motility. All microfilariae died at 48 hours, with the minimum effective concentrations (MEC) of 256 μg/ml. The effects of rifampicin (MEC = 256 μg/ml) and ciprofloxacin (MEC = 128 μg/ml) appeared later, (at day 4 and 10, respectively). The outcome of this study will be useful for treatment, and control of *D. immitis* infection and could be applied to control other human filarial parasites.

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ULTRASOUND ASSESSMENT OF SUBCLINICAL HYDROCELES IN A COMMUNITY COENDEMIC FOR WUCHERERIA BANCROFTI AND MANSONELLA PERSTANS

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Lymphatic filariasis (LF) due to *Wuchereria bancrofti* (WB) infection is endemic throughout Mali with prevalences, based on circulating antigen assays, as high as 65%. Despite this, clinical manifestations of LF occur less frequently than expected. In Sabougo, where the prevalence of infection was 48%, lymphedema, elephantiasis and hydrocele were detected in 1.6%, 0.8% and 1.6%, respectively, of the 129 circulating-antigen positive subjects enrolled in a study of doxycycline treatment of *Mansonia perstans* (Mp) WB coinfection. The purpose of the present study was to determine the rate of subclinical hydrocele in male subjects coinfected with WB and Mp in Sabougo. Only 4 of 62 subjects (6%) who underwent ultrasound examination had evidence of hydrocele on clinical examination. In contrast, 42 of 62 subjects (67%) had hydroceles (estimated volumes 10-800 ml) detectable on ultrasound exam. The “filaria dance sign” indicating the presence of live adult worms of WB was observed in 24 of 62 subjects (38%), all of whom had hydroceles and evidence of lymphatic dilatation on ultrasound examination. Subcapsular calcifications were also common, occurring in 14 subjects. These results confirm the results of studies in other endemic areas, that have demonstrated increased sensitivity of ultrasound in detecting subclinical pathology in WB-infected subjects. Although the effects of concomitant Mp infection on the clinical presentation of WB infection remain uncertain, the degree of discordance between the clinical and ultrasound examinations in Sabougo is markedly greater than that reported from areas non-endemic for Mp infection. Clinical and ultrasound examination of WB-infected male subjects from Sabougo without concomitant Mp infection should help clarify these issues.

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ONCHOCERCIASIS AND EPILEPSY IN PARTS OF THE IMO RIVER BASIN, NIGERIA: A PRELIMINARY REPORT


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The relationship between Onchocerciasis and epilepsy prevalence was investigated in an onchocerciasis endemic area in the Imo River Basin, Nigeria. Individuals complaining of seizures were identified by means of a population census in 13 villages. A total of 72 individuals were identified as possible epilepsy patients during the survey. Active epilepsy was confirmed in 58 giving a crude prevalence of 1.2%. Epilepsy was prevalent in six out of the 13 villages investigated, with highest rates in Umulolo (2.8%), Amuru (2.2%) and Akw (1.8%) and lowest in Ajaba (0.5%) and Okanachi (0.4%). The age of epilepsy patients ranged from 4 years to 59 years with majority (64%) in the 20-29 age group. The sex difference in epilepsy prevalence was not significant (p > 0.05). The prevalence of Onchocerciasis in the villages ranged from 8.3% to 36.0%. The highest Onchocerciasis rates were correspondingly found in the villages where epilepsy was most prevalent. This finding suggests a geographical association between epilepsy and Onchocerciasis. If successful control of Onchocerciasis in the area were to be followed by a fall in the prevalence of epilepsy, this may lend credibility to a causal connection between epilepsy and Onchocerciasis prevalence.
RAPID ASSESSMENT METHOD FOR PREVALENCE OF LOIASIS IN PARTS OF THE NIGER DELTA, IMO STATE, NIGERIA: A PRELIMINARY REPORT

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The prevalence of loiasis was investigated in 24 rural communities in parts of the Niger Delta Imo State, Nigeria using rapid assessment methods based on a history of eye worm (lasting 1-7 days together with confirmation by the guided recognition of a photograph of adult Loa loa in the eye) and Calabar swelling. A standardized questionnaire was administered to 1,921 individuals from randomly selected households, aged > 15 years and who had resided in the communities for at least 5 years. The results showed that the prevalence of loiasis was generally low for both diagnostic indices, history of eye worm (3.85%) and Calabar swelling (4.90%). There were insignificant differences between communities in the prevalence of eye worm and Calabar swelling (p > 0.05). Furthermore, the sex-related prevalence of history of eye worm and Calabar swelling were insignificant in males (3.02%; 3.38%) and females (5.09%, 7.03%) (p > 0.05) respectively. While the prevalence of history of eye worm was similar in all age categories, the prevalence of Calabar swelling increased with age to a peak of 15.11% in subjects 74 years and above. Both manifestations were more prevalent in farmers and traders than other occupational groups. The present findings show that rapid assessment of the prevalence of loiasis at the community level can be reliably achieved using a method based on the history of eye worm lasting 1-7 days together with confirmation by the guided recognition of a photograph of an adult Loa loa in the eye.

REAL-TIME PCR FOR THE DETECTION OF BRUGIA DNA IN BLOOD AND MOSQUITO SAMPLES

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Improved diagnostic tests for brugian filariasis are needed to support filariasis elimination efforts. We have developed two real-time PCR assays for detecting Brugia DNA in blood and mosquitoes. The highly repeated AT-rich 320 bp “18S” DNA sequence was used as a target for Taqman (TaqM, amplicon size 320 bp) or Eclipse MGB (EMGB, amplicon size 120 bp) real-time PCR assays. The EMGB assay is more sensitive than the TaqM assay and detects as few as 22 copies of the target. The EMGB assay was as sensitive as membrane filtration and microscopy for detecting B. malayi in 36 night blood samples from infected individuals in Sulawesi, Indonesia. The EMGB assay also detected parasite DNA in 17 of 31 (55%) of microfilaria-negative day blood samples from these subjects. This test was more sensitive than conventional or TaqM PCR (and almost as sensitive as night blood filtration) for detecting parasites in night blood samples from a B. timori-endemic area on Alor Island, Indonesia, where infected people had low microfilaria densities after mass drug administration (MDA). To evaluate the assays for xenomonitoring, host-seeking mosquitoes were collected on Alor Island, after 1, 2 and 3 rounds of MDA. About 25% of the collected mosquitoes were Anopheles barbirostris, the vector of B. timori, and ~75% were Culex. DNA was extracted from pools of 10 to 20 individuals and infection rates were estimated by the Poolscreen2 algorithm. Overall TaqM detected B. timori DNA in 36 of 88 Anopheles pools (infection rate 5.1%, 95% CI, 3.4-7.2%); EMGB detected 38 positive pools (infection rate 5.5%, CI 3.8-7.7%). Surprisingly, 10 and 12 of 87 Culex mosquito pools were positive by TaqM and EMGB assays, yielding estimated infection rates of 0.6 and 0.7%, respectively. Culex mosquitoes are not known to be vectors of Brugia, but microfilariae were presumably taken up with blood meals and their DNA appears to persist long enough to be detected by PCR. Our data show that real-time PCR is a sensitive means of detecting Brugia DNA in human blood and man-biting mosquitoes.

ENHANCED EXPRESSIONS OF TGF-B1 IN INFLAMMATORY CELLS, α-SMA IN STELLATE CELLS, AND COLLAGEN ACCUMULATION IN EXPERIMENTAL GRANULOMATOUS HEPATITIS CAUSED BY TOXOCARA CANIS IN MICE

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Although toxicocal granulomatous hepatitis (TGH) characterized with a dominant-Th2 type immune response is a self-limiting disease, little is known concerning the role of fibrosis-related cytokines transforming growth factor β1 (TGF-β1) in pathogenesis of TGH. A detailed histological and quantitatively immunohistochemical analysis of TGF-β1, α-smooth muscle actins (α-SMA), and collagen was performed on the liver tissues from mice infected with T. canis as assessed between day 1 and 42 weeks post infection (dpi or wpi). TGF-β1 was detected mainly in infiltrating cells in lesions with a peak at 12 wpi. Larvae per se also exhibited strong TGF-B1 expression in the trial. Alpha-SMA was detected predominantly in hepatic stellate cells (Hepatocell for Sick Children) which surrounded the lesions, reaching a peak at 28 wpi. Collagen was observed to accumulate in inflammatory lesions and biliary basement with peak content at 24 and 12 wpi, respectively. In conclusion, although enhanced TGF-1 in infiltrating cells and active Hepatocell for Sick Children with α-SMA expressions may contribute to healing of injured sites through up-stimulation of collagen deposition, abnormally persistent collagen accumulation may cause irreversible fibrotic injury in the TGH.

IMPACT OF MASS DRUG ADMINISTRATION ON THE DEVELOPMENT OF RESISTANCE IN HOOKWORM

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Hookworms infect an estimated 1.3 billion people worldwide. These parasites feed on blood, producing an iron-deficiency anemia that leads to malnutrition, stunting of growth, intellectual and cognitive retardation in children, and adversely affects intrauterine growth resulting in premature births and low birth weight. Treatment campaigns targeting hookworms and/or lymphatic filariasis using mass drug administration (MDA) have produced significant reductions in the prevalence and intensity of hookworm infections. However, mass treatment also brings with it the risk of drug resistance. Studies on several parasites of veterinary importance have demonstrated that levels of resistance are extremely high before resistance is recognized by phenotypic measures of drug efficacy. Therefore it is of critical importance to detect evolving resistance while the frequency of resistance alleles is still low and the drugs are still effective.

We are investigating the potential impact of the MDA on the development of resistance in hookworms in Haiti, where benzimidazoles have been used in the WHO-sponsored program to eliminate lymphatic filariasis. In several nematode parasites of livestock, resistance to benzimidazoles is associated with point mutations (TTT to TAT or TTC to TAC) in positions 167 and 200 of β-tubulin gene, which replace a phenyalanine (Phe) with a tyrosine (Tyr). However, mutations in other positions of the protein also may
correspond to resistant phenotypes. We therefore cloned and sequenced the β-tubulin genes of the human hookworms Anclylostoma duodenale and Necator americanus and the dog hookworm A. caninum. Mutations are being investigated using a broad range of techniques including allele specific PCR, pyrosequencing and Real Time PCR. We are also interested in investigating the population genetics of drug resistance development and spread, using microsatellites as genomic markers. Genomic libraries enriched for microsatellite sequences were prepared for all three species, and we now have set of 34 usable microsatellite markers for A. caninum.

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A RARE CASE OF TRICHURIUS TRICHURA AND HOOKWORM INFESTATION

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Heavy Trichuris trichura and hookworm infestation and surgical complications are rare in developed countries. Cases of intestinal complications including colitis, rectal prolapse, intussusceptions and perforated appendicitis with trichuriasis has been reported. We described a case of appendicular obstruction leading to necrotizing appendicitis related to heavy T. trichura and hookworm infection in a healthy young male who had emigrated to United States from Honduras six months earlier. He presented to our hospital with lower abdominal pain, fever, jaundice and vomiting for four days. He was unable to pass flatus and had no bowel movements. Abdominal examination showed rebound tenderness with guarding at right lower quadrant with sluggish bowel sounds and rectum was empty. Laboratory investigations revealed peripheral leukocytosis and neutrophilia with no eosinophilia or anemia. The liver enzymes were elevated with hyperbilirubinemia. Hepatitis Profile was negative. CT Abdomen and Pelvis revealed appendicitis with abscess, inflammatory changes of the right colon, mild intraperitoneal biliary ductal dilatation, and parasitic colonization of small/large bowel. Patient had appendectomy with abscess drainage and pathology revealed features of acute necrotizing appendicitis with perianappendicitis.

Blood culture grew Streptococcus milleri and stool sent for ova and parasite showed many T. trichura and hookworms. Patient was treated with antibiotics and Mebendazole. Patient improved and was discharged home after nine days of hospitalization. Our case reveals complicated appendicitis and cholangitis from parasitic infestation with superimposed bacteraemia. Intense local irritation, spasm of intestinal wall and increased plasma concentration of tumor necrosis factor in systemic circulation may have played a role in the pathogenesis of above manifestations.

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MOLECULAR CLONING, CHARACTERIZATION AND EXPRESSION OF CDNA DERIVED PHOSPHAGEN KINASE (PK) OF T. CANIS, A. SUUM, F. HEPATICA AND S. JAPONICUM

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Phosphagen kinases are the enzymes that catalyse the reversible transfer of the high-energy phosphoryl group of ATP to naturally occurring guaninio compounds such as creatine, glycoammine, tauroammine, lombricine and arginine, and have a key role in the interconnection of energy production and utilization in animals. In vertebrates the only phosphagen is phosphocreatine, and the corresponding phosphagen kinase is creatine kinase (CK). In invertebrates, at least six unique phosphagens (phosphoarginine, phosphoglycoammine, phosphotaurourcynamine, phosphoaurcynamine, phosphoaurcynamine and phospho-opheline) are present in addition to phosphocreatine, and the corresponding kinases for the first four such as, arginine kinase (AK), glycoammine kinase (GK), tauroammine kinase (TK) and lombricine kinase (LK), have also been identified. Here we report the isolation and characterization of cDNA derived amino acid sequences of phosphagen kinases of very important zoonic parasites such as Toxocara canis, Ascaris suum, Fasciola hepatica and Schistosoma japonicum. The phosphagen kinase can be a very good drug target site and a new approach in the control of these parasites. In this study, cDNA was synthesized from these parasites and the PK gene was successfully amplified by PCR and completely sequenced. The PK gene of S. japonicum and F. hepatica contain two domains. In S. japonicum domain I comprises 1008bp of ORF coding for a 360-amino acid residue protein and domain II comprises 1021 bp of ORF coding for a 357-amino acid residue protein. In F. hepatica domain II comprises 1071 bp of ORF coding a 352-amino acid residue protein. The cDNA of T. canis and A. suum PK comprises 1303bp of ORF coding for a 400-amino acid residue protein and 1194bp of ORF coding for a 398-amino acid residue protein respectively. The cDNA derived neucleotide and amino acid sequences of T. canis and A. suum PK placed within the nematode AK cluster while S. japonicum and F. hepatica formed a separate cluster (trematode). Further, we cloned T. canis and A. suum PK in pMD18-T plasmid vector and expressed it in E. coli as a fusion protein with maltose-binding protein.

(ACMIP Abstract)

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EPIDEMIOLOGY OF HEPATITIS C VIRUS INFECTION AND ASSOCIATION WITH HUMAN IMMUNODEFICIENCY VIRUS AMONG MEN WHO HAVE SEX WITH MEN IN LIMA, PERU

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To evaluate any potential association between hepatitis C virus (HCV) and human immunodeficiency virus (HIV), we studied 162 HIV-positive case subjects at screening and 324 age- and location-matched HIV-negative control subjects which were part of a cross-sectional HIV sentinel surveillance survey of 3,280 men who have sex with men (MSM) conducted in 6 major urban centers in Peru, during November 2002 and February 2003. Sexual behavior was assessed with a structured computer assisted self-interview (CASI); serum-based screening for HIV and HCV infections was performed by enzyme-linked immunosorbent assay (ELISA). The overall HCV prevalence in the case-control study group was 9.5%. Age-related increases and decreases were noted in HIV and HCV infection rates, respectively. HIV infection was found to be associated with HIV infection (odds ratio [OR] = 2.89), prior symptoms of sexually transmitted infections (STIs), such as urethritis (OR = 2.60) or proctitis (OR = 1.84), and a homosexual self-definition (OR = 1.80), but not with a history of illegal drug use. HCV infection among MSM in Peru is strongly associated with HIV seropositivity in a setting where injecting drug use is uncommon. STI
prevention strategies may assist in the reduction of HCV infection among
MSM in Peru.

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COMMUNITY BASED HIV/AIDS PROGRAM IN RURAL HAITI:
WHAT TO BUILD, WHAT TO BORROW AND WHERE TO BEGIN

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Haiti is the poorest and most HIV/AIDS burdened country in the Western Hemisphere (Adult prevalence rate of 5.6%). Decades of political instability, international embargoes and economic sanctions have resulted in a public health and general healthcare infrastructure inadequate to meet the most basic needs of the majority of the population. Several non-government organizations have implemented HIV/AIDS programs with notable success in select Haitian communities. However, similar to most of Haiti's population, the peasants in the rural community of Fondwa had no consistent access to HIV/AIDS education, testing, treatment or care. The formal (clinic staff) and informal (community health promoters) healthcare workers in Fondwa requested assistance in designing and implementing a community HIV/AIDS program. A current literature review on HIV/AIDS in Haiti and relevant studies on HIV/AIDS in the developing world was performed. In addition, based on principles of community based participatory research, information was obtained from: (1) A focus group consisting of representatives from the formal and informal healthcare sectors and two US based nonprofit organizations providing long-term professional, educational, financial and material support to the primary care clinic. (2) In-depth interviews with long-term Fondwa Community volunteers and the Fondwa Clinic Director. (3) Direct participant observation by the author who has been involved with a medical laboratory development project in Fondwa since 2002. Fondwa Clinic records were reviewed for general patient demographic data and results of a rapid community assessment survey were also considered. The community specific resources and needs are outlined and assessed in the current country specific context of Haiti. Recommendations for designing a model comprehensive HIV Prevention Education, Voluntary Testing and Counseling, Treatment and Care Program in rural Haiti are discussed as well as the challenges faced during the initial implementation and evaluation of the program.

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GENOTYPE DISTRIBUTION OF HIV-1 STRAINS AMONG
CHILDREN IN LIMA, PERU, 2002-05

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The Acquired Immune Deficiency Syndrome (AIDS), represents the seventh leading cause of death in young adults and the ninth in children of 1 to 4 years of age worldwide. The number of HIV infected pregnant women in Peru has increased in the past 2-3 years and vertical transmission now accounts for approximately 4% of all HIV cases reported. There is no published data regarding the distribution of HIV genotypes among pediatric populations in Peru. We examined the genotypic distribution of circulating HIV strains among children born in Lima, Peru, during the years of 2002-05. The study population consisted of HIV-infected children who were suspected or confirmed of suffering from pulmonary tuberculosis (TB) patients who were evaluated at the Instituto de Salud del Niño and/or at the Hospital Nacional Cayetano Heredia. The children's ages ranged between 6 months to 8 years old. All blood samples (n=86) that were confirmed by ELISA (EIA) and Western Blot (WB) were subjected to PCR amplification of the gag and env portions of the genome. Heteroduplex mobility assay (HMA) was performed with env PCR products followed by sequencing of the C2-V5 region of the envelope gene for undetermined samples. To gain better insight into the dynamic of the epedemics, we additionally sequenced the gag p24 and protease/RT (Pr0RT) region to monitor the prevalence of recombinant viruses. PCR Protease/RT amplified regions were sequenced using an ABI 3100 automated sequencer. A total of 84 (97.6%) were genotyped as subtype B by HMA and 2 (2.3%) of the specimens were found to be recombinant forms (CRF12_8F) representing B and F mixed genotypes by sequencing.

Co-circulation of a number of subtypes in a given population and super infection of a patient with a different virus may result in the emergence of recombinant viruses.

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HIGH PREVALENCE OF ENTEROAGGREGATIVE ESCHERICHIA
COLI (EAEC) IN AIDS PATIENTS WITH DIARRHEA IN HAITI

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Enterocaggregative Escherichia coli (EAEC) infection is a cause of persistent diarrhea in AIDS patients. The prevalence of EAEC in Haitian patients with AIDS has not been examined previously. A matched-pair cohort study (25 patients in each category) to examine antiretroviral absorption in HIV-infected patients with and without diarrhea is currently enrolling patients at the GHESKIO Centers in Port au Prince, Haiti. The patients are matched by age, sex, and CD4 count. This abstract reports preliminary results from an investigation of the prevalence of EAEC in these Haitian AIDS patients initiating antiretroviral therapy. Polymerase chain reaction (PCR) targeting the plasmid-borne aggR gene of EAEC was performed on DNA from stool samples obtained at the time of antiretroviral treatment (ART) initiation. DNA was extracted from frozen, unpreserved stool using the Qiagen DNA Stool Mini Kit according to the manufacturer's instructions. DNA was then amplified with Amplicor Gold (Applied Biosoystems) using 40 cycles of amplification (55°C annealing temperature). We were able to detect down to 10,000 cfu bacteria per gram of stool using stool spiked with positive control EAEC bacteria (strain 17-2). The presence of the aggR gene was determined by a 457 bp band visualized after electrophoresis of PCR product on a 2% agarose gel. 23 stool samples from AIDS patients with diarrhea and 9 stool samples from AIDS patients without diarrhea have been tested with this protocol. 11/23 (74%) of the patients with diarrhea and 3/9 (33%) of those without diarrhea were positive for aggR by PCR. (P = .020). Patients with EAEC by PCR in their stools had significantly higher quantitative lactoferrin (42.1 µg/ml vs 7.6 µg/ml; P = .045), and they had significantly less weight gain in the first two weeks of ART (-0.48lb vs +3.3lb; P = .039) than patients without EAEC in their stools. In conclusion, performing PCR for EAEC directly from stool samples is feasible. EAEC is significantly more prevalent in AIDS patients with diarrhea relative to AIDS patients without diarrhea in this study population. Patients with positive stool PCR for EAEC have higher levels of lactoferrin, an indicator of intestinal inflammation, and poor weight gain after the initiation of ART.

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ONE YEAR FOLLOW UP OF MOTHERS FROM THE PMTCT
PROGRAM IN ZIMBABWE: COMPLIANCE AND CHALLENGES

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This study was undertaken to describe the outcome of a one year follow up of mothers from a national PMTCT program regarding defaulters, drop outs and compliance. Nested case control study was conducted. Three peri-urban primary health care clinics in the city of Harare: Epworth, St Mary's, Seke North. Pregnant women enrolled at 36 weeks of gestation were recruited for a follow up of mother and child from delivery, 6 weeks,
4 and 9 months post partum. Follow up trend of HIV positive and negative mothers was compared regarding defaulting, drop outs, partial and full compliance. Statistical significance was computed using the chi-square test. Of the enrolled 1050 pregnant women with a known HIV status 851(81%) showed up at one or more visits scheduled up to 9 months. The denominator dropped at each point and time. The overall drop out was 19% without any significant difference between the HIV positive and negative women at delivery. The difference appeared at 6 weeks 7.7% versus 12.9% (p=0.010) and at 4 months: 2.9% versus 7.7% (p=0.002) respectively. At 9 months the drop out rate was not different (p=0.747). The default rate was significantly different at every stage between the HIV positive and negative mothers from delivery to 6 weeks becoming more significant at 4 and 9 months visits (p<0.001). Overall full compliance at 9 months was 46.1% with a significant difference between the HIV positive (55.6%) versus (37.9%) for the HIV negative (p<0.001). In conclusion, drop out is highest among the HIV negative as opposed to the HIV positive with the peak period being at 6 months. There is high defaulting among the HIV negative compared to the HIV positives with the peak period at 4 months. Full compliance is observed for the HIV positive whilst more HIV negatives complies partially. The challenge is in defining the thresholds for keeping people in the cohort and for them to fully comply with the study. There is need to assess the predictors and characteristics of the mothers that dropped out; defaulted and those that remained in the study.

THE CHARACTERISTICS, RISK BEHAVIORS AND STI PREVALENCE AMONG SOCIALLY MARGINALIZED WOMEN IN LOW-INCOME URBAN, COASTAL PERU

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This study was undertaken to describe the characteristics, risk behaviors, and STI prevalence among marginalized women in low-income, urban neighborhoods in three coastal Peruvian cities. The socially marginalized women are referred to as “movidas” (loose women) in these communities and were included in the National Institute of Mental Health Collaborative HIV/STD Prevention Trial as an ethnographic analysis of these communities suggested that they were at higher risk than women in the general population. Movidas were administered an epidemiologic survey to evaluate risk behavior and serologic tests to determine STI/HIV infection prevalence in 2001-2002. In the sample of 108 women, their mean age was 25.5 (range: 18-40), 38.9% had graduated from high school, and 56.5% had stable or occasional work while 38% were supported by their families. Their mean number of partners (standard deviation) in the past 6 months was 2.3 (7.8). Most (39.3%) had unprotected sex in the past 6 months with at least one of their past five partners and 10.5% had unprotected sex with a non-primary partner. Additionally, 27.7% of these women reported having been forced to have sex with one of their partners in the past six months. No HIV cases were found in the sample. The prevalence of HSV-2 infection was 43.0% (95% CI, 33.5% - 52.9%). The prevalence of gonorrhea was 2.8% (95% CI, 0.6% - 8.0%), Chlamydia prevalence was 18.7% (95% CI, 11.2% - 26.2%), and the prevalence of trichomonas was 6.5% (95% CI, 1.8% - 11.3%). In conclusion, movidas are a group with sexual risk behavior and STI prevalence higher than what has been found in the female general population in Peru, however they have not been included in STI/HIV prevention or control efforts. This is a highly vulnerable population with whom STI prevention interventions are warranted.

IMMUNOPATHOLOGY AND IN SITU HYBRIDIZATION IN THE DETECTION OF CUTANEOUS LEISHMANIASIS IN AN ENDEMIC REGION OF WESTERN VENEZUELA

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A parasitological, immunological and molecular diagnosis of localized cutaneous leishmaniasis (CL) was conducted from 1996 to 2004, in 27 settlements in western Merida state, an endemic Venezuelan Andes region for CL. The transmission is active, at a height of between 800 and 1800 metres in the humid mountainous zones where the anthropophagic Lutzomyia species are naturally infected. Patients were attended at the town of Tovar in Merida state site of the headquarters of the local Dermatology Service. Clinical histories revealed 5,545 infected human with 1 to 20 lesions over the forearms (10%), legs (35%), face (45%), and trunk (10%). The infection was equally common in children, women and men and the Montenegro intradermal reaction (MDR) carried out on each patient, with a dose of 0.1 ml of antigen injected intradermally on the inner surface of the left forearm was up to 29 mm in diameter. No significant differences between MDR and sex, age, number and evolution of the lesions. The relationship between the number of lesions with age and sex was non-significant. The patients received treatment quimioterapic. Anti-Leishmania antibodies for indirect immunofluorescence antibody test and ELISA was 1:400 to 1:16400. Giemsa-stained imprints of the cut of the punch biopsies pressed on a glass slide, and skin biopsies sections of seven microns stained with Hematoxin and Cosin and unlabelled peroxidase-anti-peroxidase and IFAT technique, showed inflammation site, amastigotes and Leishmania antigen. The disease was also seen in dogs and horses infected parasites were found in the nose and ulcerated scrotum and vagina of canids lesion by biopsy. The clinical presentation of the cutaneous lesions, the geographic origin of the infection and the characterization from CL lesions of the humans and domestic animals by in situ hybridization was compatible with pattern strain of L. (Viannia) braziliensis. (ACMICP Abstract)

INFECTION OF THE FETAL TISSUE IN CONGENITAL CHAGAS' DISEASE IN THE WISTAR RAT

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This work was carried out on female Wistar rats intraperitoneally injected before mating with 5x10^6 bloodstream Trypanosoma cruzi strain, and pregnant 10 days later (GI) to study the effects of acute Chagas on the fetuses infection. Rats unipregnated and injected with T. cruzi (GI), and pregnancy rats (GiI) were used as controls. Development of patent parasitemia in GI showed levels highest at the 18, 24 and 36 days post-infection (pi), with 6.12 and 20 days of gestation respectively and with significant differences at the 1% level between GI and GI. The pregnant in infected rats with T. cruzi (GI) induces specific stimulation anti-T. cruzi antibodies (Ab). Serologic test in serum samples obtained during acute phase gave positive results, with titers Ab between 1:512 and 1:2048 at the 19 and 20 days pi, and significant difference at the 5%, when groups GI and GiI were compared. T. cruzi was observed in amnionic fluid (AF) on a glass slides Giensa stained. The 33% the AF samples from rats of GI with 20 days of gestation developed trypanosomatids in humoculture NNN. Histopathological studies in sections of 6 micron Hematoxin and Cosin staining of fetal heart of 2 fetuses from rats GI with 34 days of gestation, showed parasitemia in the miocardic tissue. Placentas showed moderate placentitis, inflammatory infiltration of mononuclear and polymorphonuclear cells with abundant neutrophils. The immunotnintion with Fluorescen isothiocyanato-Propidium iodide.
and Peroxidase anti Peroxidase of placental and umbilical cord tissue of 3 rats from GI, showed intense florescence on T. cruzi antigen. These results confirmed that acute infection in gestating rats produced fetal intratuerine infection. It is possible that the massive parasite invasion in the placenta, amniotic fluid and fetal cardiac tissue is related with high maternal patent parasitemia, and a congenital T. cruzi transplacental transmission occurred early in pregnancy rats.

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IDENTIFICATION OF THE L. MEXICANA, L. AMAZONENSIS AND SUBGENUS VIANNIA BASED ON ANALYSIS OF THE RRNA INTERNAL TRANSCRIBED SPACER 2
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Human cases of leishmaniasis are caused by approximately 20 Leishmania spp., some of which are found in the same geographic region. Species identification often has clinical relevance (e.g., influences decisions about whether/which treatment is indicated). However, the gold standard method for species identification, isoenzyme analysis, requires a positive culture that remains viable long enough to yield a large quantity of parasites. Molecular approaches for species identification, using various genetic markers, are being explored. In this study, we focused on the ITS 1. We designed genus-specific primers to amplify a DNA fragment from the ITS 1 of virtually all pertinent Leishmania spp. and looked for regions in the fragment with potential utility for differentiating among species, with the ultimate goal of developing a multiplex approach. We extracted DNA from 50 isoenzyme-characterized specimens (21 skin/blood specimens and 29 cultured isolates); the species included L. (V) braziliensis, L. (V) panamensis, L. (V) guyanensis, L. tropica, L. major, and species in the L. donovani and L. mexicana complexes. We conducted PCR and DNA sequence analysis of the amplified fragments (385 to 450 bp in length). Of note, no amplification was obtained when the primers were used on DNA extracted from Trypanosoma cruzi specimens (i.e., 3 blood and 3 culture specimens). For the Leishmania specimens, DNA sequence analysis of the amplified fragments revealed complex patterns of insertions/deletions and substitutions for the various species studied. However, we found 3 regions (2 to 21 bp in length) within the fragment that, when analyzed together, allowed robust differentiation among the species. These regions may be used to design probes for a multiplex approach for species identification.

(ACMOP Abstract)

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DEVELOPMENT OF A NEW REAL-TIME PCR ASSAY TO IDENTIFY THE CAUSAL AGENTS OF LEISHMANIASIS IN PERU
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Five different species belonging to the genus Leishmania have been identified as a cause of american tegumentary leishmaniasis (ATL) in Peru, namely L. braziliensis, L. peruviana, L. guyanensis, L. lainsoni and L. amazonensis. These species present clinical outcomes that range from benign, self healing cutaneous lesions to diffuse cutaneous and mucosal ulcers. Of particular interest is the case of L. (V) braziliensis, responsible for ~80% of the cases of ATL in Peru, which is more aggressive and causes mucosal metastases in approximately 10% of the patients. The identification of the agents to the species level is of critical importance for assessing the clinical outcome, providing adequate treatment and evaluating epidemiological risks. The current gold standard for the identification of the species, Multilocus Enzyme Electrophoresis (MLEE), is time consuming and requires parasite isolation and technical expertise. We cultured 45 isolates previously typed by MLEE and sequenced the complete coding region of 4 of the main isoenzymes showing differing migration patterns between species: malate dehydrogenase (MDH), glucose-6-phosphate dehydrogenase (G6PD), glucose phosphate isomerase (MPI) and mannose phosphate isomerase (MPI). Sequence analysis revealed polymorphisms that produced nonsynonymous mutations that resulted in a shift in the MLEE patterns. As expected from phylogenetic analyses previously published, several single nucleotide polymorphisms (SNPs) were found to be unique in the four genes from L. lainsoni, L. guyanensis and L. amazonensis, whose number were directly correlated to their evolutionary divergence. Accordingly, only one SNP in MPI and two in MDH allowed the differentiation between L. braziliensis and L. peruviana. We found a subpopulation of L. braziliensis strains that showed several unique SNPs and presented different migration patterns for G6PD, GPI and MDH and that will require further investigation. Based on the SNPs identified in the MDH and MPI genes we designed a FRET based real-time PCR assay that allows a rapid, sensitive and specific identification of these Leishmania species directly from clinical samples. Because of its swiftness and simplicity it is particularly useful for use in the field and routine laboratories in endemic regions and will allow an early diagnosis and better treatment of the patients affected with ACL.

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IMPACT OF CLIMATE VARIABILITY IN THE OCCURRENCE OF LEISHMANIASIS IN BOLIVIA
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Leishmaniasis transmission occurs in many countries in the Americas. Recent reports from Venezuela and Colombia have evidenced changes in cutaneous and visceral leishmaniasis epidemiology in relation to climate variability phenomena. We evaluated the potential impact of climatic events during 1991-2000 in leishmaniasis incidence in Bolivia. Satellite climatic and epidemiological data were obtained, the last from Bolivia's Ministry of Health. NOAA climatic classification and SOI/DNI indexes were determined as global climatic variability indicators. Yearly variations comparisons and median trends deviations for disease incidence and climatic variability were obtained. Statistical analysis was performed using SPSS. Considerable climatic variability was identified during the study period, (El Niño during 5 years and La Niña for the other 5). In the same period, 16,207 leishmaniasis cases were reported, mean 1620 cases/year. During La Niña years disease incidence increased 67%, while during El Niño years there was a decrease of 40%. We found significant differences in the mean annual number of cases between La Niña and El Niño years (2029 cases/year vs. 1212 cases/year, respectively p<0.01). Linear regression demonstrated that with lower values of ONI, a higher number of leishmaniasis cases were seen (r²=0.5257, p=0.018). Higher values of SOI were associated with higher number of leishmaniasis cases (r²=0.7008, p=0.003). In conclusion, our results support the growing body of evidence that demonstrate the potential impact of climate variability in the incidence of vector-borne diseases and suggest that prevention strategies of leishmaniasis need to take into account climate variability phenomena.
ACUTE CHAGAS DISEASE IN COLIMA, MEXICO. REPORT OF A CASE AND REVIEW OF ITS EPIDEMIOLOGY

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Chagas disease still represents a relevant burden for health in many countries of Latin America. Its situation in Mexico is a matter of debate because the difficulties to document recent infection in humans in spite of extensive surveys. Here we present the case of a 17 years old boy who developed fever, lymphatic enlargement and a periorbital swelling 2 weeks before his consultation. The physical findings included Romania's sign and inoculation Chagomia in the frontoparietal area of the right side of the face, hepatomegaly and tender lymphatic enlargement in the neck. Lab examination showed moderate leucocytosis with neutrophilia and AST elevation, an echocardiogram was normal and chest X ray examination revealed moderate lung reticular infiltrates with right pleural effusion. A fresh smear from peripheral blood sample showed mobile trypomastigotes of T cruzi confirmed by Wright stain. Inoculation of newborn mice resulted in infection with significant parasitemia 2 weeks after. The patient lives in Cuahetemoc city, Colima, Mexico, a place previously reported infected with domestic triatomiene bugs. This case is the first acute documented infection in more than 20 years in Mexico and confirms our previous assumption that Chagas disease is currently an active trouble in this part of the country that deserves attention by the health authorities.

LYMPHOCYTE SUBSETS BEFORE AND AFTER SODIUM STIBOGLUCONATE TREATMENT

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Sodium stibogluconate (Pentostam®; Glaxo-Smith-Kline) is a mainstay of treatment for severe cutaneous leishmaniasis in U.S. military personnel returning from Iraq and Afghanistan. However, an increased incidence of reactivation of Varicella zoster virus (VZV) has been noted after treatment with this medication. To evaluate the effects of sodium stibogluconate on the immune system that might predispose to this adverse event, different subpopulations of immune cells were evaluated utilizing flow cytometry-based immunophenotyping in 10 patients with old world cutaneous leishmaniasis before and after 10 days of sodium stibogluconate treatment. Total white blood cells (WBCs) and total lymphocytes were analyzed along with lymphocyte subsets including helper T cells (CD3+CD4+), cytotoxic T cells (CD3+CD8+), memory T cells (CD3+CD45RO+), regulatory T cells (CD4+CD25+) and natural killer cells (CD16+CD56+). The absolute number of total WBCs decreased after sodium stibogluconate treatment by a median of 2400/mm3 (p=0.004), total lymphocytes by 800/mm3 (p=0.002), helper T cells by 265/mm3 (p=0.002), cytotoxic T cells by 159/mm3 (p=0.002), memory T cells by 221/mm3 (p=0.002), regulatory T cells by 29/mm3 (p=0.006) and natural killer cells by 54/mm3 (p=0.004). The percentage of the total lymphocyte population for each lymphocyte subset did not change significantly except for a marginal increase in percentage of cytotoxic T cells by a median of 0.77% (p=0.049). Therefore, lymphocyte subset numbers decreased overall without predilection for any particular subset. Anti-VZV antibodies were measured in 7 patients before and after treatment, and did not change (p=0.69). The general decrease in lymphocytes and especially T cell subsets may account for the increased rate of Varicella zoster reactivation in patients treated with sodium stibogluconate. Further comparison of VZV-specific T cells before and after sodium stibogluconate treatment is ongoing using an intracellular cytokine secretion assay.

PARASITE STRAIN-DEPENDENT VARIATION IN TRANS-SIALIDASE-SPECIFIC CD8+ T CELL RESPONSES IS A GENERAL CHARACTERISTIC OF EXPERIMENTAL TRYPANOSOMA CRUZI INFECTION

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The genome of Trypanosoma cruzi, the causative agent of Chagas’ disease, contains a large number of trans-sialidase (ts) genes that encode peptides recognized by CD8+ T cells in mice and humans. Experimental infection of C57BL/6 mice yields dominant CD8+ T cell responses against the ts peptides TSKB20 (ANYKTLV) and TSKB18 (ANYDFTLV). However, the kinetics and magnitudes of ts-specific CD8+ T cell responses vary...
depending on the infecting parasite strain. Peak ts-specific CD8+ T cell responses are generated earliest following CL infection (approximately D15 post-infection), and later in Brazil or Y-infected mice (D19-D24 post-infection). In contrast, the highest frequencies of ts-specific CD8+ T cells are observed in Brazil-infected mice (35% of all CD8+ T cells), followed by CL infected mice (20% of CD8+ T cells), and Y infected mice (less than 10% of CD8+ T cells). To determine whether strain-dependent differences in ts-specific CD8+ T cell responses are a general phenomenon of experimental infection, Balb/c mice were infected with Brazil, CL, or Y strain T. cruzi and responses to the ts peptide HYNQGQVSL (TSKD14) were examined. CD8+ T cells from Brazil-infected mice produced IFNγ at higher frequencies following TSKD14 stimulation than did CD8+ T cells from mice infected with CL strain. However, TSKD14-specific CD8+ T cell responses were observed earlier following CL infection (D14) than following Brazil infection (D17-24). TSKD14-specific recall responses were barely detectable from SC of Y strain-infected mice. These results document parasite strain-specific immunodominance patterns in T cell responses during T. cruzi infection. Such strain-specific patterns of responses in naturally infected hosts could allow for superinfection and could help account for differences in patterns of immune responses and disease outcomes in hosts.

(ACMCIP Abstract)

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TOLERIZATION OF ANTIGEN-SPECIFIC CD8+ T CELL RESPONSES DURING EXPERIMENTAL TRYPANOSOMA CRUZI INFECTION REVEALS REQUIREMENTS FOR IMMUNODOMINANT CD8+ T CELL SUBSETS

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CD8+ T cells are critical for host immune control of trypanosoma cruzi, the causative agent of Chagas disease. C57BL/6J mice develop a highly focused CD8+ T cell response against two epitopes derived from the trans-sialidase (ts) gene family of surface proteins, TSKb20 (ANYKFTLV) and TSKb18 (ANYDFTLV). It is unknown if immunodominant CD8+ T cell responses are required for efficient control of T. cruzi infection and pathology. To assess the requirement of these immunodominant CD8+ T cell responses, we blocked the generation of antigen-specific populations by tolerization of TSKb20-specific CD8+ T cells using intravenous injection of the TSKb20 peptide. The efficacy of treatment was monitored by assaying for the presence of TSKb20-specific CD8+ T cells in peripheral blood by staining with MHC I-TSKb20 peptide tetramers. Tolerized mice generated significantly lower frequencies of TSKb20-specific CD8+ T cells (<1% TSKb20+ CD8+ peak) compared to untreated mice (~25% TSKb20+ CD8+ peak). Mice failing to generate a strong TSKb20-specific CD8+ T cell response due to tolerance induction succumbed to infection approximately 57 days after infection. Furthermore, TSKb20 treated mice contained massive numbers of parasites in their muscle tissue as measured by histopathology and quantitative PCR. The results of this study demonstrate an immunodominant CD8+ T cell response focused on ts-derived epitopes is a critical element in control of T. cruzi infection. Ongoing studies are aimed at addressing the relative contribution that sub-dominant CD8+ responses make to the immune control of T. cruzi.

(ACMCIP Abstract)

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VACCINATING AGAINST TRYPANOSOMA BRUCEI USING RECOMBINANT VIBrio CHOLERAE GHOSTS EXPRESSING TRYPANOSOMAL CA2+ PUMP PROTEIN

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Trypanosoma brucei spoto causes human African Trypanosomiasis (HAT, sleeping sickness) disease with a reported estimate of 50,000 new cases and equal number of deaths in Sub-Saharan Africa. Early symptoms of African Trypanosomiasis include fever, headaches, and joint pains. If treatment is not sought after several weeks, the parasite can then cross the blood-brain barrier to invade the nervous system which can result in coma and death. This severe epidemic calls for the development of an efficacious vaccine that is capable of protecting against infection. Past studies have shown the development of vaccines against HAT to be unsuccessful because of the parasites ability to evade the host immune system by antigenic variation due to its expression of distinct variable surface glycoproteins. We have designed a novel vaccine delivery system that is capable of eliciting antibodies against their epitope. Calcium ATPases have been identified and are localized in the less dense flagellar pocket where they function to maintain cytosolic calcium ion concentration for the survival and proliferation of the parasite. Therefore, we hypothesized that using recombinant Vibrio cholerae ghosts (vCG) as a vaccine delivery vehicle expressing trypanosomal TBCA2, a calcium ATPase, will inhibit the function of these cation pumps preventing parasite survival. To test our hypothesis naïve mice were vaccinated, thrice at two-week intervals, with vCG expressing TBCA2 or phosphate buffered saline. Two weeks following the last vaccination mice were challenged with T. brucei and observed for survival and parasitemia. Also splenic T-lymphocytes were harvested from mice to assess the induction of TNF-α, INF-γ, and IL-10. T-lymphocytes were cultured for five days in the presence or absence of the TBCA2 peptide. Supernatants were then collected and analyzed for TNF-α and INF-γ, pro-inflammatory cytokines, and IL-10, anti-inflammatory cytokine. Although vaccinated mice tolerated higher parasitemia they were able to survive longer and expressed higher levels of TNF-α than the control group. Analysis of INF-γ and IL-10 are in progress. Administering vCG-TBCA2 as a vaccine increased mouse survival and can therefore, be used as a novel approach in the development of a vaccine against human African Trypanosomiasis.

(ACMCIP Abstract)

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RUNT DISEASE LIKE SYNDROME CAUSED BY LEISHMANIA PARASITES IN THE MURINE MODEL

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Runt disease in mice is a syndrome characterized by several clinical features agreed upon by most researchers. All the reports point to the same symptoms in fact the animals become wasted losing their fur with cutaneous atrophy, loss of subcutaneous fat, muscle volume, and even bone mass. These subjects start shrinking very gradually to become significantly smaller in size compared to their non-injected littermates. Furthermore diarrhea if it develops would be an additional symptom to all of the above health problems. The pathophysiologic changes are identical with the features that characterize graft versus host disease. The pathologic findings on post mortum examination reveal invariably an enlarged spleen and liver. Abscences in the liver are often observed. We report the changes that take place in animals (inbred Balb/c mice)
susceptible to Leishmania parasites injected with promastigote forms of this parasite grown in axenic cultures, collected and injected at the log phase of their growth. The course of illness starts a few weeks after the animals have received the inoculum. The gradual changes observed clinically and the time frame for the full blown picture of running are described. The animals are sacrificed when their survival for even a few hours becomes doubtful. Our contention that these parasites are the cause of these changes is substantiated by several findings, beside the clinical picture. They comprise the gross findings at autopsy, in addition the histologic studies of the samples from the skin, the liver and the spleen support our suspicion. Finally the cultures showed no bacterial growth beside the expected parasitic growth. These results are discussed in view of what has been reported in the literature on runt disease in mice whether caused by a graft attacking the host or by particularly virulent microorganisms such as Salmonella Typhi or as in this report Leishmania in Balb/c mice.

(ACMIP Abstract)

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CIRCULATING CELL-DERIVED MICROPARTICLES IN MALARIA PATIENTS

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Activation of vascular endothelium and blood cells in a range of inflammatory and infectious diseases is associated with the formation of cell-derived microparticles (MPs), which are membrane vesicles with diameter <1.5 μm. Here we describe the quantities, the cellular origin and the possible pathogenesis of MPs in malaria. Using flow cytometry, we found that the median (range) of circulating MPs was increased in patients with falciparum malaria (N=29, 2051 (222-43280) μl), p<0.01), vivax malaria (N=5, 840 (376-1141) μl), p<0.01) and malariae malaria (N=2, 499 (499-500) μl, p<0.01) compared to healthy controls (N=11, 163 (81-375) μl). Patients with severe falciparum malaria showed higher numbers than uncomplicated falciparum malaria (N=19, 2567 (366-43280) μl) versus (N=10, 1947 (222-4107) μl), p<0.01). The number of MPs rapidly declined after start of antimalarial treatments and remained below 800 μl after 48 hours of admission. MPs were mainly released from red blood cells (RBCs), platelets (PLTs), and endothelial cells (ECs). In order to study the effect of oxidative malaria haem products on MP formation, we exposed RBCs, PLTs and human brain endothelial cells (HBECs) to haem (12.5-100 μg/ml). Above 50 μg/ml haemien bleb formation on RBCs could be observed electron microscopically, and this correlated with an increase in the red cell derived microparticles (RMPs) numbers and a decrease in RBC surface diameter. This was not observed in PLTs and HBECs. Haemien induced RMP formation was inhibited by the antioxidant N-acetylcysteine (1 mg/ml). To establish RMP formation from P.falciparum-infected RBCs, we measured RMPs in the supernatant from synchronous culture. It was found that most RMPs were produced during schizogony. Altogether, this suggests that MP formation is increased during malaria infection and is associated with severity of disease. Formation of RMPs might result from haemien induced oxidative stress and schizogony.

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THE BURDEN OF MALARIA INFECTION ON PREGNANT WOMEN AND THE INFANTS

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Malaria infection is one of the major problems encountered by pregnant women in the tropical region. Its severity reduces with increase gravidity and age of the mother. This study investigated the effect of gravidity and age of the mother on the severity of malaria infection. It is also aimed to show the burden of infection on the haemoglobin level of the mother and the birth weight of the infants born to them. Peripheral blood was collected from 262 pregnant women who attended Ade-Oyo maternity hospital, Ibadan, Nigeria. Of the pregnant women studied, 128 and 134 were primigravidae and multigravidae respectively. Thick blood smears were prepared for parasite identification and quantification. Anaemia was detected by measuring Ht levels using Drabkin's solution. Age, gravidity, gestation, and history of treatment with antimalaria drugs were obtained from the pregnant women using questionnaires. The overall prevalence of infection was 41.8%. The prevalence of infection was higher in primigravidae (35%) than multigravidae (22%). Teenagers and primigravidae were more infected than the adults. Of the pregnant women studied, 76.4% were anaemic and this was associated with increase in parasitaemia. The severity of the anaemia was significantly higher (p<0.05) among malaria positive teenagers and primigravidae than adults and multigravidae. The mean birth weight of infants born to malaria positive was significantly lower (p<0.05) than those born to malaria negative mothers. Malaria positive teenagers and primigravidae had children with lowest birth weight as compared with adult and multigravidae. This study suggests that the prevalence of malaria infection and anaemia were higher among teenagers and primigravidae than adults and multigravidae. Malaria positive mothers had babies with very low birth weight than malaria negative mothers. Age and gravidity also affected the birth weight of the infants.

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PLASMODIUM FALCIPARUM CULTIVATION USING THE PETRI DISH: REVISITING THE EFFECT OF THE 'AGE' OF ERYTHROCYTES

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The Petri dish method is one of the most popular methods of cultivating the parasite Plasmodium falciparum. Differences in the characteristics of the culture conditions can influence the multiplication rate for this organism. In previous studies, ideal culture conditions for the growth of these organisms was achieved by using erythrocytes collected from blood that had been stored at least 2 weeks. In the present study, we studied the multiplication rate for P. falciparum in cultures containing erythrocytes of various "ages". In vitro multiplication rates for P. falciparum decreased as the duration of erythrocyte storage increased. This trend was consistent despite the interval of medium changes, which ranged from 12 to 48 hours. On the fourth day after inoculation (i.e., 2 life cycles), the parasitemia in the culture containing erythrocytes that had been stored for 28 days was approximately half that of the parasitemia in the culture containing fresh erythrocytes. When the medium was changed every 12

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hours, the first 2 weeks of storage turned out to be critical, as indicated by the fact that the multiplication rate for *P. falciparum* decreased more steeply in cultures during the first 2 weeks of erythrocyte storage than the subsequent two weeks. However, when the medium was changed every 48 hours, the multiplication rate decreased more steeply in cultures during the latter period of erythrocyte storage. When the medium was changed every 24 hours, the multiplication rate decreased regularly along with the increase of erythrocyte storage duration. The results of this study strongly suggest that “younger” erythrocytes are better than aged ones for cultivating *P. falciparum.*

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**CYTADHERENCE OF PLASMODIUM FALCIPARUM STRAINS FROM SINGLE AND MULTIPLE GENOTYPE INFECTIONS FROM SYMPTOMATIC CHILDREN IN FRANCEVILLE, SOUTH-EASTERN GABON**

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The virulence of *Plasmodium falciparum* is due to its ability to induce severe malaria by sequestration of parasitized red blood cells (PBMC) in the microvasculature of major organs such as the brain. The objective was to investigate whether the level of cytadherence is influenced by *P. falciparum* multiple genotype infection (MGI) and to determine the relationship between cytadherence and disease severity. The nested polymerase chain reaction was used to genotype *P. falciparum* isolates and identify SGI (single genotype infection) and MGI from symptomatic children. Cytadherence of PBMC was determined using the in vitro model of human lung endothelial cells (HLEC). Genotype analysis of two highly polymorphic regions of the merozoite surface antigen (MSA1) and (MSA2) and a dimorphic region of the erythrocyte binding antigen (ERA-175) revealed 9/42 (21.4%) SGI and 33/42 (78.6%) of MGI in symptomatic children. Cytadherence varied from 58 to 1,811 PBMC/mm² of HLEC for SGI and from 5 to 5,744 PBMC/mm² of HLEC for MGI. There was no significant difference between mean cytadherence in SGI (1021) and MGI (1028). No association was observed between cytadherence levels and severe malaria (p=0.92). However, the K1 genotype of the MSA1 locus was observed in 82% of isolates from individuals with severe malaria. These results showed that cytadherence to HLEC was not influenced by the multiplicity of clones in *P. falciparum* infection and confirmed field evidence of an association between severe malaria and a specific genetic characteristic of the parasite.

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**IN VITRO CULTURING OF PAPA NEW GUINEAN *P. VIVAX* FIELD ISOLATES USING UMBILICAL CORD RETICULOCYTES**

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*Plasmodium vivax* (PV) has proven difficult to grow in culture and therefore thorough study of this parasite has been limited. Nine years ago, using the PV Chesson strain, Golenda et al. successfully improved existing short-term PV culturing techniques by periodic supplementation with reticulocytes obtained from a hemochromatosis patient. Blood samples from this donor (3-5% reticulocytes) were processed by ultracentrifugation to enrich reticulocytes to a final working concentration of 10%. This technique, while useful, is labor intensive and relies upon frequent donation of blood from a patient with an uncommon blood disorder. In PNG PV is endemic. To facilitate *in vitro* PV culturing umbilical cord blood from post partum mothers (3-8% reticulocytes) provides an ideal alternative source of reticulocytes to supplement *P. vivax* cultures. Furthermore, we have developed a technique that further enriches reticulocytes harvested from cord blood to 70%. In our studies we observed that a one-time addition of 20 uL of the reticulocyte solution at the initiation of cultures in 2 mLs McCoy’s 5A Medium +20% Human AB serum at a 5% hematocrit (PV starting parasitemia 18%-68%) enabled significantly longer (p<0.002) PV viability (8 days, ± 0) compared to cultures initiated in media alone (4.25 days, ± 0.95). These results suggest that regular supplementation of PV cultures will allow us to extend *in vitro* PV cultures for multiple rounds of blood-stage replication. These studies will enable us to evaluate mechanisms of PV erythrocyte invasion and drug susceptibility.

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**INCREASED SEVERE ANEMIA IN HIV-1-EXPOSED AND HIV-1-POSITIVE INFANTS AND CHILDREN DURING ACUTE MALARIA**

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Since the primary hematological complication in both pediatric HIV-1 and malaria is anemia, co-infection with these pathogens may promote life-threatening severe malarial anemia (SMA). The primary objective of the study was to determine if HIV-1 exposure (HIV-1(exp) and/or HIV-1 infection (HIV-1(in)) increased the prevalence of SMA in children with acute malaria. The effect of HIV-1 exposure and HIV-1 infection on the prevalence of SMA (hemoglobin less than 6.0 gr/dL), parasitemia (parasites/µL), high density parasitemia (HDP; >10,000 parasites/µL or greater) was investigated in children < 2 years of age presenting at hospital with acute *Plasmodium falciparum* malaria in a rural holoendemic malaria transmission area of western Kenya. Upon enrollment, a complete hematological and clinical evaluation was performed on all children. Malaria parasitemia was determined and children with acute *P. falciparum* malaria were evaluated for HIV-1 exposure and infection using two rapid serological antibody tests and HIV-1 DNA PCR, respectively. Relative to HIV-1(-) group (n=194), the HIV-1(exp) (n=101) and HIV-1(+)(n=23) groups had lower hemoglobin concentrations (P<0.001 and P<0.001, respectively), while parasitemia and HDP were comparable between the three groups. Multivariate analyses, controlling for age, gender, and sickle-cell trait demonstrated that the risk of SMA was elevated in HIV-1(exp) children (odds ratio, 2.17; 95% CI, 1.25-3.78; P=0.01) and HIV-1(+) children (odds ratio, 8.71; 95% CI, 3.37-22.51; P<0.0001). The multivariate model further revealed that HIV-1 exposure or infection were not significantly associated with HDP. Results presented here demonstrate that both HIV-1 exposure and HIV-1 infection are associated with increased prevalence of SMA during acute *P. falciparum* infection, independent of parasite density.

(ACMCIP Abstract)
MOLECULAR BASIS OF *PLASMODIUM FALCIPARUM* RECEPTOR BAEBL FOR BINDING TO ERYTHROCYTE LIGAND GLYCOPHORIN C

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*Plasmodium falciparum* invades human erythrocytes by redundant pathways. Unlike *P. vivax* that has one Duffy Binding-Like (DBL) receptor, *P. falciparum* has four members of the DBL receptor family. Furthermore, one of these DBL genes, BAEBL, has polymorphisms at four amino acids in region 2, the receptor region of the protein; each polymorphism binds to a different red blood cell (RBC) ligand. One BAEBL variant (VSTK) binds specifically to erythrocyte glycophorin C. BAEBL (VSTK) is the only one that had threonine at amino acid 121 (T121) in place of K or R. We modeled the structure of region 2 of BAEBL (VSTK) on the crystal structure of a related DBL receptor, EBA-175. Four charged amino acids, Arg 52, Arg 114, Gln 54 and Asp 125, are predicted to surround T121 on the model of BAEBL (VSTK). They were individually mutated to alanine and expressed on the surface of CHO cells. The wildtype binds poorly to Gerbich negative cells that have a deletion of exon 3 in glycophorin C. In contrast, the mutations in arginine 52 or 114 caused reduced binding to normal RBCs and had similar binding to Gerbich negative RBCs. Mutations of glutamic acid did not affect binding, that is, they still bound normal RBCs at the same efficiency and have markedly reduced binding to Gerbich negative RBCs. These findings suggest that the two arginine residues surrounding T121 are critical for RBC binding and may be critical for sialic acid binding.

(ACMCIP Abstract)

REDUCED STEM CELL GROWTH FACTOR PRODUCTION IS ASSOCIATED WITH THE DEVELOPMENT OF CHILDHOOD SEVERE MALARIAL ANEMIA

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In holoendemic *Plasmodium falciparum* transmission areas, such as sub-Saharan Africa, severe malarial anemia (SMA) is the leading cause of morbidity and mortality in malaria-infected individuals. The underlying causes of SMA are multifactorial and include direct and indirect destruction of parasitized and non-parasitized red blood cells (RBC), inefficient erythropoesis, and dyserythropoesis. Upon resolution of a malaria infection, new RBC production is essential for recovery from SMA. Although many soluble factors, such as erythropoietin, increase the erythropoietic response, it is currently unclear if decreased production of these factors contributes to the development of SMA. Stem cell growth factor (SCGF), a recently discovered hematopoietic growth factor, possesses burst-promoting activity for human bone marrow erythroid progenitors. Since no study to date has reported a role for SCGF in malaria, circulating SCGF levels were determined in children with varying severities of malarial anemia (*n*=128), and the relationship between SCGF, hemoglobin (Hb) concentrations, reticulocyte production index (RPI), and parasitemia were examined. Children with SMA (Hb <6.0 g/dL) had reduced circulating SCGF that was positively correlated with Hb levels (*r*=-0.213 *P*<0.019) and the RPI (*r*=-0.316 *P*<0.016). SCGF was not significantly associated with parasitemia. Peripheral blood mononuclear cells (PBMC) from children with malaria cultured under baseline and stimuli-induced conditions further revealed that children with SMA produced lower SCGF under baseline conditions (*P*<0.05).

In vitro experiments with PBMC from healthy, malaria-naïve U.S. adults demonstrated that malarial pigmentation (hemozoin, pfHr) and synthetic PfSCGF transcript expression (*P*<0.05). Moreover, circulating SCGF in children with malaria was negatively correlated with the amount of pigment-containing monococytes (*r*=-0.325 *P*<0.013). Taken together, these results illustrate that naturally acquired PfHr decreases mononuclear cell SCGF production and that reduced SCGF may be an important hematopoietic factor that contributes to the development of SMA.

REAL-TIME BEDSIDE MEASUREMENT OF NITRIC OXIDE DEMONSTRATES IMPAIRED PRODUCTION IN ADULTS WITH SEVERE MALARIA IN PAPUA, INDONESIA

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Nitric oxide (NO) has been associated with protection from severe malaria in both children and adults. However, NO has a half-life of seconds and studies of the role of NO in malaria have been hampered by the difficulty in measuring production during disease. Most studies have measured concentrations of NO metabolites in body fluids. The results of many of these studies have been difficult to interpret because of the failure to control for dietary nitrate ingestion, nitrate retention in renal failure and perturbations in fluid volumes. Studies that have controlled for these variables have shown reduced levels of NO metabolites in severe disease. Other studies have shown impaired expression of NO synthase in circulating mononuclear cells in severe malaria. However, to date, there have been no real-time direct measurements of NO itself in malaria. In this longitudinal study conducted at Mitra Masyarakat Hospital in Timika, Papua province Indonesia, adult patients (age 18-60 years) with uncomplicated and severe malaria underwent serial bedside measurement of exhaled NO in parts per billion (ppb) using the NIOX apparatus and American Thoracic Society Guidelines. Measurement required the ability to sit and to cooperate with the exhalation technique and was not possible in subjects with cerebral malaria or prostration. Baseline measurements were possible in 60 patients with moderately severe malaria (patients requiring inpatient parenteral therapy but without WHO manifestations of severe malaria) and 12 with modified WHO criteria for severe malaria. Median exhaled NO was lower in severe malaria [10.5 ppb (IQR: 9.5-15.0)] than moderately severe malaria [18.5 ppb (IQR: 11.1-26.9); *p*=0.03]. By 48 hours, exhaled NO in patients with severe disease had increased to levels comparable to those found in healthy controls (median 16.6 [IQR: 11.9-27.0]). Real-time bedside measurement of exhaled NO allows direct measurement of NO production in malaria, and demonstrates impaired production in patients with severe malaria compared to those with moderately severe malaria. Results are consistent with a protective role for NO in malaria. Measurement of exhaled NO has potential utility in evaluating interventions targeting increased NO production in severe malaria.
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PYRUVATE KINASE DEFICIENCY PROTECTS AGAINST PLASMODIUM FALCIPARUM MALARIA

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In malaria-endemic regions, Plasmodium falciparum infection constitutes a great challenge for the population. Epidemiological studies have demonstrated a correlation between malaria disease and genetic mutations, in particular, patients with hemoglobinopathies were shown to be protected against severe malaria. Two principal mechanisms have been postulated to explain this observation: first, the inhibition of invasion and growth of parasites within mutated erythrocytes (E), and second, the enhanced clearance of parasitized mutant E. After glucose-6-phosphate dehydrogenase deficiency, Pyruvate kinase deficiency (PKD) is the most frequent enzyme abnormality of the glycolytic pathway, and the most common cause of hereditary non-spherocytic hemolytic anemia. Recently PKD in mice was shown to be protective against malaria. Whether PKD protects against Plasmodium falciparum remains unknown. We show here two strains of P. falciparum that the parasite invaded less PKD E than normal E. We also found that membrane-bound hemichromes, IgG and complement C3c fragments bound, and phagocytosis by human and mouse monocytes/macrophages were higher in ring-stage in PKD E than in normal E. Nevertheless, when P. falciparum parasites invade PKD E their growth is similar as in normal E. Reduced invasion and enhanced uptake of ring-parasitized PKD E may low the parasite load in the blood of PKD patient and protect them against severe malaria.

(ACMCIP Abstract)

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DISRUPTION OF CS CONFRS RESISTANCE TO CEREBRAL MALARIA

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Host genetic factors are important in determining susceptibility to cerebral malaria (CM) in both humans and in mouse models. Infection of known resistant and susceptible inbred mouse strains with Plasmodium berghei ANKA provides a good model system to identify host genetic determinants that regulate the development of CM. Complement component 5 (C5), which sits at the crossroads of the classical and alternate pathways of the complement system, has been implicated in susceptibility to other infectious diseases. Since activated C5 (C5a) participates in pro-inflammatory cascades, we hypothesized that it may contribute to the inflammatory cytokine-associated encephalopathy that characterizes human and murine CM and that C5 deficiency would confer protection. To examine this hypothesis, 5xI/0 P. berghei ANKA parasites were injected into a panel of mouse strains, mice were genotyped at the C5 locus and were monitored for survival and parasitemia. Infected mice were found to exhibit significantly different survival curves and could be divided into four groups: very susceptible (e.g. 125SV), susceptible (e.g. C5B6R/6 or B6), resistant (e.g. A/J) and very resistant (e.g. AKR); however, all susceptible and very susceptible mice were wild type (WT) at the C5 locus. Transfer of the C5-defective allele from A/J onto a susceptible B6 genetic background conferred resistance to CM. Conversely, a congenic A/J strain containing the WT C5 allele from B6 mice were susceptible to CM. Additionally, in the closely related B10.D2/NsSnJ and Osn, which differ only at their C5 locus, the C5 deficient mice had higher survival rates and lower parasitemia than WT mice. These data provide direct evidence that C5 contributes to the development of CM in the P. berghei ANKA model, suggesting a role for C5 and complement pathways in human CM.

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EFFECT OF SUPHADOXINE-PYRIMETHAMINE ON ANTIOXIDANT DEFENSE SYSTEM AND LIPID PEROXIDATION

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Due to the spread of resistance to chloroquine by Plasmodium falciparum. Sulphadoxine-pyrimethamine (SP) became a cheap alternative drug of choice for the treatment of malaria in most endemic areas. Production of oxygen radicals forms part of the host defense and pathology of malaria. Exacerbation of intra-erythrocytic oxidative stress might contribute to the process of elimination of the parasites. The effect of treatment with SP on the antioxidant defense system was investigated using rabbit as a model.

Ten male rabbits were divided into two groups of 5 animals each. The first group was administered with normal saline and served as control. The second group received a single dose of SP (26.25mg/kg body weight). Blood samples were collected before and at 6, 12 and 24 hr after saline or drug administration. Activity of cellular enzymatic antioxidants, superoxide dismutase (SOD) and catalase (CAT), and reduced glutathione(GSH) were assayed using standard photometric methods. Serum lipid peroxidation was assessed by the formation of malondialdehyde while protein content was assayed by the method of Lowry.

SOD activity was observed to increase progressively by 4.9, 63.4 and 120.8% at 6,12 and 24 hr respectively after drug administration. Similarly, CAT activity increased by 44.5, 82.6 and 116.3% at 6, 12 and 24 hr respectively. Malondialdehyde levels also significantly increased by 45.5, 118.2 and 186.4%. However, the activity of GSH was observed to have decreased by 41.9% by 6hr and remained so till the 12th hour, but by 24 hours after drug administration, the activity has increased significantly up to 48.4% above the 0hr level. SP treatment altered the enzymatic antioxidant defense system and lipid peroxidation in blood and therefore could induce oxidative stress. The increase in SOD activity is an indication of generation of reactive oxygen species.

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MICROFLUIDIC PLATFORMS FOR MALARIA PATHOGENESIS

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Severe malaria caused by the parasite Plasmodium falciparum is a potentially fatal disease, in part due to the failure of host organs brought about by the accumulation of parasitized red blood cells in the microvasculature. Severe disease symptoms are associated with capillary blockage due to the loss of deformability of infected erythrocytes, their sequestration to endothelial cells and their interaction with the host immune system. Despite much progress in understanding disease pathophysiology from postmortem autopsies and in vitro adhesion assays, there remains a need for experimental systems to study the pathogenesis of the disease in a controlled, multicellular environment that closely mimics capillaries. We show here that microfluidic devices with dimensions similar to those found in real capillaries can be engineered to grow and support both host cells and infected red blood cells under conditions of continuous fluid flow for time periods of up to 24 hours. Using these devices, we have been able to measure novel characteristics of sequestration of parasitized RBCs to cells and recombinant proteins such as CD36 and ICAM-1 in channels resembling narrow capillaries, and phagocytosis of infected red blood cells that are continuously exposed to fluid shear forces. The development of these microfluidic tools that mimic capillaries in the body can therefore shed light on how infected RBCs interact with different host cells in vivo. The devices also have the potential to be a valuable, portable research tool in malaria endemic areas.
OPTIMIZATION OF A HEPATOCYTE CULTURE SYSTEM FOR IN VITRO SCREENING OF COMPOUNDS AGAINST LIVER STAGES OF P. FALCIPARUM AND P. VIVAX

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After mosquito injects malaria sporozoites into human body, the parasites firstly invade liver cells to proliferate and subsequently invade red blood cells. Advancement of knowledge regarding liver stage parasite development has been slower than in other developmental stages due to the lack of a simple in vitro or in vivo model. Development of effective antimalarial drugs against liver stages of human malaria will require an efficient in vitro system because this cannot be done in humans. We have established a new human hepatocyte line, HC-04, that supports the exo-erythrocytic (EE) development of both Plasmodium falciparum and P. vivax. To establish an in vitro system for screening of new compounds against liver stage malaria, this system is being optimized to increase sporozoite invasion and production of key proteins/enzymes in the hepatocyte cell line. Different culture conditions and parasite preparation methods have been compared. We have measured levels of different forms of CYPh450 that are involved in drug metabolism and major protein production such as albumin, etc., using RT-PCR and quantitative PCR. Plasmodium falciparum or P. vivax sporozoites were inoculated into the HC-04 cell line and cultured under three different culture media, i.e., MEM-F-12, Hepatocyte Culture Medium (HCM, Cambrex), or DMEM-F-12 supplemented with Hepatocyte Growth Factor (HGF). Identification and quantification of the parasite development was accomplished using microscopic examination, quantitative PCR, and measurement of fluorescence of FITC tagged parasites using HIV-1 Tat protein-transduction domain. We also compared the gene expression profiles of major liver proteins and drug metabolizing enzymes among different cell passages and among parasite infected and non-infected cells using RT-PCR. This model will be further validated for in vitro screening of compounds against liver stages of P. falciparum and P. vivax once the system is optimized.

PYRUVATE KINASE DEFICIENCY PROTECTS AGAINST PLASMODIUM FALCIPARUM MALARIA

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A number of epidemiological studies have reported a correlation between malaria-exposure and selection for a variety of genetic erythrocyte disorders. In particular, several hemoglobinopathies have been reported to protect against severe and fatal malaria. Two principal mechanisms have been postulated to explain this observation: the inhibition of invasion and growth of parasites within altered RBCs and second, enhanced clearance of parasitized variant RBCs. After glucose-6-phosphate dehydrogenase deficiency (G6PDH), pyruvate kinase deficiency (PKD) is the most commonly recognized erythrocyte enzmyopathy. Recent work by Min-Oo, et al. demonstrated that PKD is protective against severe and fatal malaria in a murine model (Min-Oo et al, Nature Genetics, 2003). However, whether PKD might be protective against Plasmodium falciparum malaria is unknown. In this study we investigated Plasmodium falciparum invasion, intracellular growth, and clearance of infected PKD RBCs versus normal erythrocytes. We demonstrate, using multiple Plasmodium falciparum clones, that there is an invasion defect for PKD RBCs compared to normal erythrocytes (Inv: 3.7±2.7 vs 10.8±9.9, p = 0.0002). We also show significantly increased membrane-bound hemichromes (0.162 vs 0.038 nmol/ml RBC membrane), IgG (0.669±0.011 vs 0.097±0.02 absorbance unit/min/10^6 RBCs, p < 0.001) and complement C3c fragments (1.66±0.017 vs 0.125±0.005 absorbance unit/min/10^6 RBCs, p < 0.001) on infected PKD RBCs as well as increased phagocytosis by human and mouse monocytes/macrophages of ring-stage PKD RBCs compared to infected normal RBCs (phagocytic index 29.9±8.3 vs 2.1±0.44 %, p = 0.003). Reduced invasion and enhanced uptake of ring-stage parasitized PKD RBCs may contribute to a lower parasite burden in PKD patients and confer protection against severe malaria.

EMERGENCE OF NEW GENOTYPES AND INCREASES IN DOMINANT GENOTYPE COPY NUMBER ARE ASSOCIATED WITH DEVELOPMENT OF SYMPTOMATIC MALARIA IN THE VILLAGE OF MISSIRA, MALI

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Previous PCR-based studies of polymorphic loci, such as Block 2 of merozoite surface protein 1 (msp1), have shown that Plasmodium falciparum infections have greater complexity in areas with high transmission intensity. In order to further study the role infection complexity plays in disease, we followed a cohort of children from 2 to 10 years of age with monthly filter paper blots using a combination of real-time PCR based on Block 2 of msp1 (allele-specific copy number) and capillary electrophoresis (genotype identification based on amplicon size) to define the genotypes present and the copy numbers for each genotype. To test the hypothesis that changes in parasite genotype and genotype copy number were associated with the development of disease, we identified 29 subjects who developed disease between September and November 2005 (symptomatic malaria = positive smear for asexual P. falciparum parasites plus fever, headache or other symptoms or signs of uncomplicated malaria) following an asymptomatic infection (positive smear, absence of clinical symptoms or signs), within a cohort of 401 children. In terms of genotype, 15 of the 29 children who developed disease had a new dominant genotype at the time of their illness, while 14 retained the same dominant genotype (p > 0.05). In terms of copy number, 16 of the 29 subjects who developed disease had a ≥ 50% increase in the copy number of the dominant genotype (p > 0.05). However, 24 of the 29 subjects either developed a new dominant genotype or had a (p = 0.001). These results suggest that development of disease in areas with high transmission may be driven both by infection with new genotypes and by increases in the copy number of the dominant genotype.

RELATIONSHIP BETWEEN THE NEUROTOXICITY AND PHARMACOKINETIC PROFILES OF ARTEMISININ DERIVATIVES IN ANIMAL SPECIES

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Artemisinin has been identified as the active antimalarial component of qinghaosu, a plant used for centuries to treat malaria in China. Artemisinin, and its analogues dihydroartemisinin (DHA), artether (AVI), arteether (AS), artesunate (AS), and arteline (AL), are highly effective against the erythrocytic stages of Plasmodium falciparum in vivo and in vitro. In the past decade, in vivo toxicity studies have showed a dose-dependent neurotoxic effect associated with movement disturbances, spasticity, brain tissue damage, and even death in animal species with the
oil-soluble artemisinin derivatives AM and AE. In contrast, few neurotoxic
effects have been shown to be induced by the water-soluble artemisinins
AS and ALD. Early studies have never induced any CNS side-effects in
animal species after intravenous injection of AS or AL. To date, however,
neurotoxicity has not been convincingly demonstrated in humans treated
with any of the artemisinins. Pharmacokinetic profiles demonstrate that
fatal neurotoxicity was caused by the oil-soluble artemisinins (sesame oil
vehicle) as the drug depot in the intramuscular injection sites resulted in
a long drug exposure time due to the slow and prolonged absorption in
the muscle and accumulation in the blood. The mild and light toxicities
induced by the water-soluble agents in contrast were found to be a result
of their very short half-lives and lack of accumulation. In conclusion, drug
exposure time has been demonstrated to play a more important role in
producing neurotoxicity than drug exposure level, production of the more
active metabolite (DHA), or having the drug located in the CNS system.
Therefore, appropriate dose regimens with the correct formulations are
necessary in avoiding neurotoxicity in humans and in animal species.

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POTENTIAL TOXICITY OBSERVED IN CHILDREN WITH
ACUTE UNCOMPICLATED FALCIPARUM MALARIA
WHILE ON TREATMENT WITH INCREASED DOSES
OF CHLORPHENIRAMINE PLUS CHLOROQUINE COMBINATION

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This study was undertaken to compare the safety of two regimens of
chlorpheniramine plus chloroquine combination in the treatment of acute
uncomplicated falciparum malaria in children. Ninety-nine children aged,
0.5 - 14 years, with acute uncomplicated malaria were randomised into
two treatment groups. Forty-eight children were allotted to the high dose
chlorpheniramine-chloroquine group and 51 children to the higher dose
chlorpheniramine-chloroquine group. The children in the high dose group
received 14 - 20 mg of chlorpheniramine daily for 7 days in combination
with chloroquine 30mg/kg orally over 3 days while the higher dose group
received, 20 - 28 mg of chlorpheniramine daily for 7 days in combination
with chloroquine 30mg/kg orally over 3 days. Outcome measures were
vital signs, clinical response and parasite clearance all of which were
monitored on days 0-7 and day 14. The vital signs though showed no
significant differences in both groups with respect to the pulse, systolic
and diastolic blood pressure, the respiratory rate was significantly lower
in the higher dose group relative to the high dose group on day 2 (28
± 4 versus 33 ± 7 cycles/min, P=0.004). Drowsiness was also commoner
in the higher dose group. The measures of therapeutic response showed
no significant differences in the two treatment groups. The parasite
clearance time was 2.8±0.7 days and 2.9±0.7 for the high dose and
the higher dose group respectively (P=0.58), fever clearance time was
1.4±0.7 and 1.3±0.7 days respectively for the high dose and the higher
dose group (P=0.68) and cure rate was 95.8% and 94.1% respectively
(P=0.94). In conclusion, these data suggest that even though these
combinations of chlorpheniramine with chloroquine may be generally
safe and effective, the higher dose chlorpheniramine -chloroquine had no
therapeutic advantage over the high dose. However, the lower respiratory
rate observed in the higher dose chlorpheniramine plus chloroquine
combination calls for caution in the clinical application of the combination.

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IN VITRO PHARMACOKINETICS OF PYRIDONE DERIVATIVES

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4H)-pyridone derivatives are a family of antimalarial agents that act as
potent selective inhibitors of Plasmodium falciparum mitochondrial
function by blocking the electron transport chain. These derivatives show
nanomolar IC₅₀ against Plasmodium falciparum and have also been proven
efficacious in mouse models of malaria. Although in vitro IC₅₀ and IC₅₀,
are excellent predictors of the potency of an antimalarial, they provide
essentially no information on the time course of antimalarial activity
and on wether killing can be increased by higher drug concentrations.
In order to determine the pharmacodynamic properties of pyridones,
time-kill studies were performed with representative members of this
family. For these experiments we used the 3DTA and FC3R3 P. falciparum
strains. Artemether, atovaquone, chloroquine and pyrimethamine were
included as representative drugs with different rates of action. IC₅₀ were
determined by the standard sigmoidal fit in vitro continuous method. Pyridone
derivatives showed IC₅₀ values from 1 to 10 ng/ml. Time-inhibition curves
were performed at concentrations equivalent to 10, 30, 100, 300 and
1000 times the corresponding IC₅₀ values. At predetermined time points,
aliquots were taken, the corresponding drug was removed and the
cultures were tested for [³H]-hypoxanthine incorporation. Experiments
were performed in duplicate. In all the cases, pyridone derivatives demonstrated
time-dependent antimalarial activity. Inhibition of parasite growth
increased gradually with the time of exposure, reaching 90% inhibition
after 15-24hrs of treatment. Saturation of the inhibition rate occurred
by ten times the IC₅₀, drug concentrations above these values do not
inhibit parasites significantly faster or more extensively. According to this
pattern of inhibition, the goal of a dosing regimen for pyridones would be
to optimize the time that serum levels exceed some minimal value such as
the IC₅₀. Further in vitro and in vivo PKPD studies will be required to
demonstrate this point.

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POPULATION PHARMACOKINETICS OF MEFLOQUINE FOR
MALARIA PROPHYLAXIS IN AUSTRALIAN SOLDIERS

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The population pharmacokinetics (PK) of mefloquine (MQ) were
determined in Australian soldiers on peace-keeping duties in East Timor
following weekly prophylaxis with MQ (Lariam). Data was obtained from
two clinical trials, a double-blinded randomised study (group A), and an
open-label study (group B). Following a loading dose (250 mg MQ) daily
for 3 days for group A, and 250 mg MQ every second day on 3 occasions
for group B), the subjects received an oral weekly maintenance dose of
250 mg MQ for 6 months. The PK study consisted of 1111 soldiers (group
A: 162 subjects; group B: 949 subjects) who had a mean (range) age
of 26 years (18-55) and weight of 82 kg (52-135). Blood samples were
collected after the last loading dose, in weeks 4, 8, and 16 for group A,
and in weeks 13 and 26 for group B. HPLC was used to measure plasma
MQ concentrations. Population PK modelling was performed using
NONMEM. A linear, two-compartment model with first-order absorption
and interoccassion variability (IOV) on the clearance of MQ best described
the data. The typical population PK parameter values were as follows:
 clearance (CL/F): 2.09 L h⁻¹; central volume of distribution (V/F): 528 L;
absorption rate constant: 0.240 h⁻¹; inter-compartmental clearance (Q):
12.5 L h⁻¹; peripheral volume of distribution (Vp): 483 L; terminal half-life:
14.0 days. Body weight had a small proportional influence on V/F but
was insufficient to warrant any alteration to dosing. The interindividual
variability (coefficient of variation, CV%) for CL/F and V/F was 24.4%
and 29.6%, respectively. The IOV for CL/F was 17.8%. The proportional
residual unexplained variability component (CV%) for groups A and B
was 11.5% and 19.5%, respectively, while the additive component (SD)
was 57 µg L⁻¹ and 149 µg L⁻¹, respectively. This is the first population
pharmacokinetic model that describes the disposition of mefloquine when