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

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

We compared 5 groups of children and their colonizion with resistant phenotypes of bacteria not receiving any antibiotic, antifungal or antitubercular agents 1 month before positive culture for resistant phenotype (A), receiving TMP/SMX only (B), receiving TMP/SMX with any antibiotic, antitubercular or antifungal agents (C) and receiving any antibiotic, antifungal or antitubercular agents without TMP/SMX (D).

Children not receiving any antibiotic had similar occurrence of phenotypes of multiresistant bacteria or Candida spp. than those receiving TMP/SMX prophylaxis (B) or receiving only ATB treatment plus TMP/SMX prophylaxis (C) or ATB and EB treatment alone (D). Of 62 children colonized and exposed to TMP/SMX and other antibiotic, antifungal or antitubercular agents, 36% isolates were MRSA, 27% Candida spp., 15% were ESBL producing Enterobacteriaceae and 14% penicillin resistant pneumococci.

Assessing prior exposure with one antibiotic only, there was no major difference of a particular pathogen relationship to any particular antibiotic. When comparing children with prophylactic antibiotic therapy with or without TMP/SMX, those children colonized with fluconozol resistant Candida spp. had significantly higher prior exposure to fluconozol with or without TMP/SMX (P<0.0004). Candida albicans was also significantly associated with prior exposure to ciprofloxacin (P<0.0004) with or without TMP/SMX.

When comparing children after TMP/SMX prophylaxis plus other antibiotic therapy, 3 significant associations appeared: MRSA and AMO+TMP/SMX exposure (P<0.04). Candida albicans and CIP+TMP/SMX exposure (P<0.03), NAC FLU-R and fluconozol exposure (P<0.0006). Comparing previous exposure of ATB plus TMP/SMX versus exposure of TMP/SMX only, no differences between both groups have been observed - TMP/SMX does not contribute to less or more resistance in MRSA, PRF, ESBL producing Enterobacteriaceae.

429

EFFECTIVENESS OF ARTEMETHER - Lumefantrine in unsupervised Home Treatment of uncomplicated malaria

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

Community based drug distributors (CBDs) in 7 villages in Oke-Ara Local Government, Oyo State, Nigeria were trained on signs and symptoms of malaria, use of Coartem®, and preparation of thick blood film. The CBDs in turn trained their community members on these topics. Treatment guidelines were distributed to CBDs and households in the communities. Follow-up study of children aged 4 - 59 months presenting with fever to CBDs began in October, 2005 and still on-going. Thick blood films for microscopy and Whatman filter paper for PCR analysis were prepared by CBDs from each patient at presentation (DO) and on Day 28 (D28) for those parasitaemia on D0. Laboratory assistants collected slides daily for screening and results were provided to the CBDs within 24 hours of collection. Caregivers were interviewed on D28 on how drug was administered and occurrence of adverse event. Two hundred and eighty seven patients have been enrolled to date and 209 (72.8%) had parasitaemia at D0. 131 of the 209 (62.7%) patients reported for D28 follow up and 54 of them had patent parasitaemia - D28 cure rate of 58.8%. The PCR analysis is yet to be completed. Eighty-two of 131 (62.6%) mothers who reported on D28 were interviewed. Seventy four (59.2%) of them reported they used Coartem® correctly and only 9 (11.0%) reported adverse event in their children. All the mothers found the guidelines useful and were of the opinion that the drug was effective. A failure rate of 41.2% is above figures reported in clinical trials using Coartem® in Nigeria and Africa and is a source of concern for a drug that is yet to be introduced for widespread use in the community. However, some of the cases with parasitaemia at D28 may be re-infections especially as a high drug dosage compliance rate was recorded by mothers. PCR analysis of D28 samples would be valuable in distinguishing re-crudescent infections from re-infections especially in this area of intense malaria transmission. Provision of adequate IEC materials stands to increase compliance to correct use of

430

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

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

431


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

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DOSE DEPENDENT EFFICACY OF SP FOR INTERMITTENT PREVENTIVE THERAPY OF MALARIA IN PREGNANCY AMONG HIV INFECTED ZAMBIA WOMEN

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WHO recommends 2 courses of intermittent presumptive therapy (IPT) with sulfadoxine-pyrimethamine (SP) for reducing malaria in pregnancy during trimesters 2-3. Monthly SP is superior to standard IPT for HIV+ women; the optimal number of SP courses is uncertain. This is an "as treated" analysis of a recently completed placebo controlled, double-blinded study comparing standard 2-dose SP-IPT (S-IPT) vs. intensive monthly SP (I-IPT) among HIV infected women from Ndola, Zambia. Although participants were randomized to either S-IPT or I-IPT, subjects actually received from 1-6 courses of SP (1500 mg sulfadoxine/75 mg pyrimethamine) by study end. We compared outcomes between 1 vs. >2 courses of SP. Outcomes were maternal hemoglobin (Hb), placental infection (% positive histology), birth weight (grams), low birth weight (LBW) (% <2500 g), (DBH1), infant cord blood parasitemia (% positive), and prematurity (% gest. age <37 weeks by ultrasound). 391 women contributed data; placenta were obtained for 360 (91.4%). Treatment groups were similar in regards to age and gravidity. 34 women received 1 course of SP; 357 ≥ 2-courses; 178 ≥ 3; and 122 ≥ 4. Additional courses of SP showed graded benefits for all outcomes (Table); lower birth weight and infant prematurity were strongly associated with single course SP 40.3% of babies born to mothers who had 1 course were LBW vs. 10.1-11.3% who had 2 or more courses (RR 3.6, 95% CI 2.2-6.0, P<0.001). 1 dose; 2 or more; 3 or more; 4 or more: Maternal Hb - g/dl (n) 10.5 (30) 11.4 (321) * 11.4 (154) * 11.5 (105) * Placental infection - % (prop.) 3.3 (10/323) 2.6 (68/329) 2.5 (41/161) 2.0 (26/113) Infant birth weight - grams (n) 2642 (32) 2962 (345) * 2985 (171) * 3015 (119) * Infant cord blood parasitemia - % (prop.) 6.9 (2/29) 1.8 (6/331) 0.6 (1/163) * 0.9 (1/115) * Gestational age < 37 weeks - % (prop.) 82.4 (28/34) 54.6 (195/357) * 53.9 (96/178) * 47.5 (58/122) * In conclusion, though our intent-to-treat analysis revealed no differences between S-IPT and I-IPT, this "as treated" analysis showed dose-dependent benefits of SP courses to mother and baby. Single course SP is inferior to multi-dose regimens and should not be used, even in areas with mild malaria transmission. Given the inferiority of single course SP, programs must ensure that HIV+ pregnant women at risk of malaria receive at least 2 IPT courses of SP. (* P<0.05 for comparison vs. 1 dose of SP. ** P<0.01).

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

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Laboratory diagnosis of parasitic diseases typically relies on identification of diagnostic morphologic features of parasites. The accuracy of morphology-based diagnosis depends on laboratorians' expertise in recognizing such features of the organism and the quality of the specimens being examined. Between 2002 and 2005, the parasitology reference diagnosis laboratory at the Centers for Disease Control and Prevention accepted 2598 specimens for diagnostic or confirmatory assistance. Approximately 21% of those requests were received at CDC as telediagnosis inquiries through the DPDX project; telediagnosis is the electronic transmission of digital images of suspected parasites in clinical specimens. To perform telediagnosis a laboratory must have a microscope, a digital camera, and a computer with Internet access. In this study we report data on DPDX telediagnosis activities from 2002-2005. During this period, DPDX processed 513 telediagnosis inquiries for diagnostic assistance, of which an average of 64% did not require additional examination or further testing for definitive identification of the parasite associated with the infection. An average of 22% required follow up by direct morphologic examination of the specimen through microscopy, and an average of 14% required PCR to achieve final identification of the parasitic agent at species level. The 160% increase in telediagnosis consultation during this period indicates a positive trend in the use of telediagnosis as a diagnostic tool in parasitology. The cost and time effectiveness of using telediagnosis for assistance will also be reported by comparison with the traditional ways of handling specimens. Telediagnosis is approximately 80% less expensive and faster, as diagnostic assistance can be provided within minutes to hours through telediagnosis. We conclude that because parasitology remains a highly visual field, use of a remote technology approach such as telediagnosis can be extremely useful in confirmatory diagnosis.

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


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Insecticide treated bed nets (ITNs) are an effective malaria control tool throughout Sub-Saharan Africa. Policy makers and donor partners need better evidence regarding which ITN distribution system is the most efficient and sustainable. In order to generate comparable and policy-relevant evidence we aimed to review systematically five large national-scale ITN programmes: targeted subsidized distribution through antenatal clinics (ANC) in Malawi; free distribution at the community level in Eritrea, EPN - linked free distribution in Togo; public-private partnership in Senegal; and finally an integrated national programme based on public-private partnerships from 2002-2005. During this period, ITN distributed through Reproductive and Child Health clinics in Tanzania. Operational descriptions of the five national ITN programs in Sub-Saharan Africa were
compiled using a standardized framework. Costs were compiled for both the set-up and maintenance phases, using a standard costing framework developed for this purpose on the basis of existing guidelines. The emphasis was put on a financial analysis of the cost to the provider, but a full economic analysis was also performed. Further analysis will include a cost-effectiveness analysis using the effectiveness data from an existing systematic review. Results indicate that there are significant differences in cost per output (cost per ITN delivered, cost per person-year protection) for different strategies but that all distribution systems are highly attractive public health interventions by any standard. Start-up costs are usually high due to the need for extensive training and intense promotion. Main sources of variation in cost were related to the type of targeting and to the cost of the nets. In conclusion, all different approaches lead to rapid increase in the level of protection afforded to the main groups at risk. Determinants of cost and implications for financing, health system capacity strengthening, equity and sustainability need to be further investigated.

435

REVERSIBLE LYMPHATIC DYSFUNCTION CAUSED BY GNATHOSTOMA SPINIGERUM INFECTION

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

436

THERAPEUTIC RESPONSE TO THIABENDAZOL, IVERMECTIN AND ALBENDAZOLE IN IMMUNOCOMPROMISED AND IMMUNE COMPETENT PATIENTS INFECTED WITH STRONGYLOIDES STERCORALIS

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The objectives of this study were: 1) Compare efficacy of thiabendazole (TBZ), ivermectin (IVM) and albendazole (ALB) in immunocompromised and immunocompetent hosts infected with S. stercoralis. 2) Identify risk factors associated to an inadequate therapeutic response. 292 patients with Strongyloidiasis were studied between 1989 y 2005, 193 immunocompetent and 99 immunocompromised hosts (38 HIV/AIDS and 61 with other immunosuppressive disorder (melioporferative disorders, lymphoma, solid tumors and/or steroid therapy). 175 cases received TBZ (119 immunocompetents, 21 HIV/AIDS y 35 non-HIV immunocompromised hosts), 83 IVM (43, 15 y 25, respectively) y 34 ALB (31, 2 y 1, respectively). At enrollment, they underwent through clinical history and physical examination and provided stool specimens for identification of larvae through Baermann technique and agar plate culture. Parasitological cure: 88.4% (260/294), TBZ: 91.1%, IVM: 96.3% and ALB: 58.6%. There was no statistical difference between TBZ and IVM treatment for Strongyloidiasis (p=0.516). Patients who received IVM or TBZ were more likely to have parasitological cure than those who received ALB (OR\(_{\text{TBZ}}\) 50, IC 95% 2.4-1073; p=0.002). (OR\(_{\text{TBZ}}\) 184; IC 95% 3.3-10.211; p=0.001). Patients with eosinophil counts <450/mm\(^3\) at diagnosis associated to high parasitological burden (≥50 larva pc) were less likely to have parasitological cure (OR 0.02, IC 0.015-0.027, p=0.011). Similarly, among patients with high parasitological burden (≥50 larva pc), those who have eosinophil counts ≥ 450/mm\(^3\) at diagnosis were 13.5 times more likely to have parasitological cure than those that had < 450 eosinophils/mm\(^3\) at diagnosis. In conclusion, TBZ and IVM are both good options for the treatment of Strongyloidiasis. HIV patients responded better to IVM than to TBz, but there was not statistical difference. ALB is an inadequate treatment for Strongyloidiasis. Patients without eosinophilia, HIV/AIDS and other immunocompromised patients, have a higher chance of treatment failure when the parasitological burden is high.

437

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

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Porridge is a primary weaning food for children in Botswana. Because fortified weaning foods can prevent malnutrition, a national program has provided free vitamin A-fortified sorghum-soy porridge to all children < 5 years old since 1993. In response to a severe pediatric diarrhea and malnutrition outbreak in Botswana, we assessed the use of porridges in the community. From March 29 - April 12, 2006, we used two-stage random cluster sampling to survey households with children < 5 years. Caregivers were interviewed about child health, diet history, and perceptions and use of fortified porridge; children were weighed and measured. We defined acute malnutrition as weight-for-height Z-score ≤ -2 or edema/kwashiorkor, and stunting as height-for-age Z-score ≤ -2. We examined porridge use for associations with diarrhea and malnutrition. We assessed 339 households with 537 children < 5 years old. The median age was 28.3 (range 0-59) months; 265 (49.5%) were male. During the 24 hours before interview, 394 (74.1%) children had consumed any porridge and 58 (10.9%) had fortified porridge distributed by the

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fleeing program. Since January 1, 2006, 170 (32.0%) children had at least one diarrhea episode, 35 (8.0%) were acutely malnourished, and 67 (15.5%) were stunted. Diarrhea, acute malnutrition, and stunting were not significantly associated with eating fortified porridge 24 hours or 7 days before interview. Among caregivers, 187 (58.8%) considered fortified porridge beneficial to children. Of those who did not, 102 (82.9%) believed it causes diarrhea and 11 (8.9%) that it makes children ill. Adult consumption of government-distributed fortified porridge intended for children <5 years old was reported in 247 (78.7%) households. In conclusion, despite free distribution, fortified porridge was regularly consumed by only one-tenth of children <5 years old and did not appear to protect against diarrhea or malnutrition. Many caregivers did not consider fortified porridge beneficial to children; instead, it is frequently consumed by adults. Further evaluation of fortified porridge distribution, preparation and practices may identify opportunities to improve the national feeding program.

438

CLINICAL DIFFERENCES BETWEEN IMMUNECOMPETENT AND IMMUNECOMPROMISE PATIENTS INFECTED WITH STRONGYLOIDES STERCORALIS

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The objective of this study was to compare clinical symptoms and eosinophil count in immune competent and immune compromised patients infected with Strongyloides stercoralis. 635 Strongyloides cases were studied from 1989 to 2005, 398 were immune competent patients and 237 immune compromised (65 HIV/AIDS and 142 with malnourished patients, lymphomas, solid tumors and steroid therapy). At enrollment, they underwent physical examination and laboratory and provided stool specimens for identification of larvae through Baermann technique and age plate culture. Two retrospective studies were conducted comparing: 1) Immune competent patients vs. HIV/AIDS, and 2) Immune competent vs. Immune compromised patients without HIV infection. HIV patients were less likely to present neither relative nor absolute eosinophilia (OR_0.001 = 0.1; IC95%: 0.1-0.3; p<0.001), and, OR_0.001 = 0.1; IC95%: 0.1-0.2; p<0.001). Furthermore, they are more symptomatic than immune competent hosts (OR=6,4; IC95%: 1.9-21,0; p<0.001). Strongyloides in HIV/AIDS patients is strongly associated to diarrhea (OR=4,8; IC95%: 2.2-10,6; p<0.001), chronic diarrhea (OR=4,5; IC95%: 2.0-10,5; p<0.001), loose stools (OR=15,4; IC95%: 1.9-125,0; p<0.001), more than 5 stools per day (OR=3,5; IC95%: 1.1-10,8; p<0.001) and weight loss (OR=6,4; IC95%: 3.2-12,6; p<0.001). Non-HIV/AIDS immune compromised patients complain less of abdominal pain and are less likely to develop eosinophilia (OR_0.005 = 0.4; IC95%: 0.2-0.8; p=0.005) coma (OR_0.001 = 0.4; IC95%: 0.2-0.8; p=0.011). In conclusion, S. stercoralis is an important cause of diarrhea and wasting syndrome in HIV/AIDS patients in developing countries. Almost 40% of HIV/AIDS patients do not develop eosinophilia ≥ 5% and 64.6% have < 450 eosinophils/ mm3. This parasite should be suspected in immune compromised hosts coming from endemic areas even in absence of eosinophilia. IC95%

439

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

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

440

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

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

441

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

442

LEISHMANIASIS AND HIV CO-INFECTION IN NORTHCENTRAL VENEZUELA
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Leishmaniasis species can cause a wide spectrum of cutaneous disease in HIV-positive patients: from cutaneous (CL), mucocutaneous (ML), diffuse cutaneous or post-kala-azar, and also visceral leishmaniasis (VL). We evaluated the epidemiologic features of leishmaniasis in a series of HIV-positive patients from Northcentral Venezuela from 2000 to 2006. A total of 6 patients with the clinical diagnosis of leishmaniasis and HIV-infection were evaluated at our referral center. Different diagnostic methods were used to confirm the diagnosis (Montenegro skin test, MST; indirect Immunofluorescence Test, IFT; and smear). Clinical and epidemiologic features of leishmaniasis among these patients were evaluated. Mean age was 26 years of age, (range 3 to 41 years); 83% were males and 17% were females; 33% were from Sucre State, with a mean clinical evolution of 8 months. From these patients, 66% (4/6) were CL, 17% (1/6) were ML and 17% (1/6) were VL. From those patients with CL, most of them presented just one lesion (75%, 3/4), all of them were anergic to MST. Co-infection of HIV and leishmaniasis species in Northcentral Venezuela is rare. However, it tends to be associated with other disseminated cutaneous or visceral leishmaniasis. The use of skin testing for leishmaniasis is not clinically helpful in patients with HIV-infection.

443

THE WIDAL TUBE DILUTION TEST EVALUATION AMONG TYPHOID FEVER PATIENTS IN JAKARTA, INDONESIA.
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Microorganism isolation through culture is the gold standard in diagnosis of typhoid fever. However this procedure takes two to seven days. In the interim the attending physician requires some laboratory evidence in the management of the patient, sometimes with high fever and altered mental status. To evaluate the value of single Widal test, we conducted a serological evaluation of 129 febrile patients admitted into a community-based prospective study of enteric fever in North Jakarta, Indonesia. The culture testing of these patients showed that they included 67 typhoid fever patients, 3 paratyphoid A patients and 1 patient with a positive blood culture for Salmonella group C; the rest had negative blood culture.

Using a cut-off of ≥1:40 for O antigen, the Widal test had 72% sensitivity, 82% specificity, positive predictive value of 82% and negative predictive value of 71%; the corresponding values for the H antigen were 35% sensitivity, 100% specificity, 85% positive predictive value and 55% negative predictive value. At a cut-off of ≥1:80, for O antigen, this test had 51% sensitivity, 96% specificity, positive predictive value of 94% and negative predictive value of 62%; the corresponding values for the H antigen were 35% sensitivity, 100% specificity, 100% positive predictive value and 57% negative predictive value. There was no cross reactivity of Widal test results, except that 2 patients with S. paratyphi A positive culture tests had positive values of 1:160 and 1:160 for O antigen. Early management of typhoid fever in specific populations may benefit from a single Widal test in the acute phase, if an appropriate cut-off tier for that population has been determined.

444

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

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

**445**

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

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Hepatitis E Virus (HEV) causes large epidemics of acute and sporadic hepatitis in Asia, the Middle East, and parts of Africa and Mexico. Epidemics are frequently associated with fecal contamination of drinking water. Death due to fulminant hepatitis may result from HEV infection and the mortality rate ranges from 1-2% among the general population to 20-30% in pregnant women. Currently there is no vaccine commercially available for hepatitis E. Several outbreaks of hepatitis E have been reported in military environments which led to significant loss of soldier duty days. Between October 99 and December 2005, Thai soldiers were deployed to Timor-Leste (n = 5 140), Afghanistan (n = 109), Iraq (n = 879) and Burundi (n = 174) as part of multinational forces for the United Nations in its peace keeping efforts. Effective disease surveillance does not exist in any of these countries. The objective of this study was to determine the antibody pattern to HEV in serum over time using a sensitive EUSA technique. Cases of hepatitis E were not reported during the deployment. Background prevalence of anti-HEV in this military population was 19.6, 20.2, 21.3 and 28.3% for troops deployed to Timor-Leste, Afghanistan, Iraq, and Burundi, respectively. The seroconversion (as defined by a 4-fold rise in antibody titer) rate per year was 1.9, 5.6, 5.6 and 4.8% respectively. These were not significantly different. A vaccine to protect against hepatitis E would be an important adjunct to prevent this important disease which can cause high morbidity among military personnel.

**446**

**STUDIES ON SOME NUTRITIONAL FACTORS IN THE SEVERITY OF VISCERAL LEISHMANIASIS**

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

**447**

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

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

**448**

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

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In 1999, baseline data from children aged 6-59 months treated with sulfadoxine/pyrimethamine (SP), in a 14-day in vivo test, revealed only 7% treatment failure at Butimba sentinel site in Mwanza, Tanzania. The
data together with other from 7 more sites were utilized in changing first line antimalarial drug from chloroquine to SP in 2001. The current study to assess the efficacy of SP, amodiaquine (AQ), and Artemether/ Lumnefrin (Coartem®) was conducted at Bumtumba in 2005. The main aim was to obtain more data on Plasmodium falcerum response to the 3 drugs. Coartem® is earmarked to become first line antimalarial drug in Tanzania in 2005. Children who met the inclusion criteria were randomized to receive one of the 3 drugs (SP=64, AQ=64, Coartem® =83). Filter paper bloodspots were collected for PCR parasite genotyping. SP was given as a single dose equivalent to 1.25mg/kg body weight with respect to pyrimethamine whilst AQ was given in 3 divided doses totalling 25mg/kg body weight (over three days). All daily doses of SP and AQ were supervised. The first dose of Coartem® and the daily doses for the next 2 days were administered under supervision. Successful 28 day follow-up was made in 61, 62, and 74 cases on SP, AQ, and Coartem® respectively. Evaluation of data based on 28 day follow-up showed adequate clinical and parasitological response (ACPR) of 54.1%, 80.6% and 83.8% in the SP, AQ and Coartem® treatment arms respectively. There were no cases of early treatment failure (ETF) with Coartem® whilst highest ETF rate was in the SP arm at 18.0%. Overall late treatment failures (LTF) were: SP=27.9%, AQ=17.7%, Coartem®=16.2%. By day 14 of follow-up, ACPR was 99%, 91.9% and 97.3% in the above treatment arms, indicating that most failures occurred later on. These results with other data have been used in reviewing anti-malarial drug policy in Tanzania. We conclude that SP is no longer effective and Coartem® is a promising alternative in the area. AQ should be considered in fixed dose combination. PCR corrected data to confirm true LTF cases will be presented.

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


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Bednets and intermittent SP therapy protect young African children from malaria infection, illness and death. Quantitative evidence of these benefits is not easily acquired. We hypothesized that bednet use would also be associated with better/faster growth in this age group, and that curative treatment during the high malaria transmission period would further enhance health and development. Random sampling of 640 children 6-24 mos. old in a non-irrigated sector of northern Ghana during August, 2001, found no evidence that bednet use was associated with fewer/lower parasitemias, higher Hb, or better Z scores for growth. Non-fibrile breast-feeding children of informed, consenting parents were enrolled and randomized to receive either supervised SP + quinine curative treatment (n=251) or a vitamin D placebo (n=247). Malaria infections, Hb, and anthropometric measures were recorded at baseline and at regular intervals. During 16 weeks of follow-up (Aug.-Dec. 2001) slide-confirmed malaria accounted for 68% and 70% of illnesses, respectively, in the treated and placebo groups. Between group comparison at endpoint showed an expected delay to first symptomatic parasitemia in the “covered” group but an unexpectedly better profile of SP effect of treatment of uncomplicated malaria cases in the placebo group. Within group comparison between baseline and endpoint identified significant improvements in Weight-for-Age (WAZ) and Height-for-Age Z (HAZ) scores in both groups that were independent of bednet use or initial cure status. Children in HAZ and WAZ quartiles and those below median HAZ were improved significantly over that of boys. Mean Weight-for-Height Z scores, WAZ, and HAZ of enrolled children were significantly improved over those of a closely matched cohort of non-enrolled children. Children in both study arms achieved improved Hb and growth independent of bednet use or malaria treatment. Concurrently, despite rapidly falling Anopheles biting rates, non-enrolled children experienced lowered Hb levels, heightened parasitemia, worsened Z scores, and greater mortality. Study participation was protective and beneficial in both treatment and placebo groups.

MICROSHERE ASSAY FOR RELIABLE IDENTIFICATION OF CRYPSTOSPORIDIU M HOMINIS AND CRYPTOSPORIDIU M PARVUM IN STOOLS

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

IMPAIRED ABILITY TO DOWN MODULATE THE IMMUNE RESPONSE

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Leishmania brasiliensis is the causal agent of cutaneous leishmaniasis (CL) and mucosal leishmaniasis (ML). CL is characterized by one or multiple ulcers and ML is a severe complication of CL involving the nasal mucosa, palate and pharynx. The protective immune response to leishmania spp is mediated by IFN-γ and TNF-α production and induction of microbicidal products by macrophages. However, the immune response may also play an important role in the pathology of the disease. In the present study we related the immune response, as determined by measurement of cytokines in supernatants of mononuclear cells, as well as cellular activation markers of tissue damage. Moreover, the mechanisms involved...
in the exaggerated type 1 immune response observed in CL and in ML were evaluated. A correlation was found between production of pro-inflammatory cytokines and severity of clinical infection. There was a direct correlation between IFN-γ as well as cell markers of activation and increase in lesion size. The exaggerated T cell response in CL and ML was associated with a strong type 1 immune response as well as IL-17 production. This biased immune response was not appropriately modulated by IL-10 and CTLA-4. IL-10 fail to inhibit TNF-α and IFN-γ production in CL and ML patients and expression of IL-10 receptor was decreased in ML. This study extends our previous observation on the participation of pro-inflammatory cytokines and T cells in the pathogenesis of CL and ML. Furthermore, it supports the use of antimony combined with molecules that down-modulate the immune response in the treatment of CL and ML.


452

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

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


453

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

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

The results of this comparative study should be of value for laboratories that plan to implement molecular tools for laboratory diagnosis of cyclosporiasis.

(ACMOP Abstract)


454

TICKS INFECTED WITH RICKETTSIA IN VILLETA, CUNDINAMARCA, COLOMBIA

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In 1993, an outbreak of Rocky Mountain spotted fever occurred in Tobía, Cundinamarca, Colombia. At that time, three species of hard ticks (Ixodidae) were known as vectors of the disease, namely Rhipicephalus sanguineus, Dermacentor nitens, and Amblyomma cajennense. However, in 2000, one genus of Argasidae (Ornithodorus) was considered the most likely vector of Tobía fever despite the fact that rickettsiae were not identified in any of the specimens collected. Since then, no tick-directed studies have been conducted in the area. We recently reported three

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

(ACMCP Abstract)

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

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**458**

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

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Aguirre, Sophie Colombani, Sandrine Gulia, Brice Madeline,
Florence Petiard, Jean Lang

Sanofi Pasteur, Marcy-L’Etoile, France

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

(ACMOP Abstract)

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**459**

**LARVAL COMPETITION AFFECTS DENGUE VIRUS INFECTION IN ADULT Aedes aegypti AND A. albopictus**

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

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**460**

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

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Dengue virus (DENV), the etiological agent of Dengue Fever, is transmitted
to the human host during the blood uptake of an infective vector
mosquito by the injection of infectious saliva. DENV dissemination into
mosquito salivary glands is therefore an essential event for the vector
to become infective. In the present study, we investigated the presence
of proteins able to bind all four DENV serotypes in either Aedes aegypti
(L.) or Aedes polynesiensis (Mark) salivary gland extracts (SGE). We also
extended our experiments to Aedes albopictus C6/36 cell extracts, this
cell line being frequently studied as an in vitro model for virus/mosquito
cell interactions. Using Virus Overlay Protein Binding Assay (VOPBA)
we detected several DENV-binding proteins, some might be implicated
in virus attachment and/or entry into mosquito C6/36 cells or salivary glands.
Since this last event is necessary for the virus to be transmitted into
the host, our study paves the way for the identification of target proteins
that would be key elements for new “DENV transmission-blocking strategies”.

Otherwise, since SGE not only contain salivary gland tissue-stemmed
proteins but also salivary ones, our study bring first elements for
the identification of “DENV/vector salivary protein” complexes, those might
interact with host immune agents at the earliest step of human infection.

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**461**

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

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In Peru, dengue was first documented in the city of Iquitos, in 1990. Since
then dengue has become endemic in many regions. In Puerto Maldonado
5 patients with positive serology for Dengue were reported in October
2001 and one dengue isolate was obtained in 2004. Here we describe
the April to June 2005 dengue outbreak in Puerto Maldonado, Peru. The
objective of this study was to describe the number of patients affected
with dengue during April to June 2005 in Puerto Maldonado. And to
describe the signs and symptoms, as well as their duration, and the age
group in which the outbreak appeared. 198 patients older than 5 years
were evaluated in the city of Puerto Maldonado. All patients had an
acute febrile illness. Acute and convalescent phase blood samples were
collected and the identification of the etiologic agents by cell culture
isolation and serological assays were performed. Of the 198 patients,
51 were diagnosed with dengue: 11 by seroconversion and 40 by virus
isolation. The serotype of the isolated viruses was dengue-3. The age
of the patients ranged between 9 and 60 years. The average age was
29 years and 22 were males and 29 females. The signs and symptoms
in the acute phase were: fever in 51 patients (100%), malaise in
51 patients (100%), chills in 45 patients (88,2%), headache in 45
patients (88,24%), retro orbital pain in 43 patients (84,31%) and myalgias
in 41 patients (80,39%). The remaining signs and symptoms are listed in
this presentation. 44 patients were asymptomatic during the convalescent
evaluation and the average of days of symptoms was 5. No patients
presented symptoms compatible with hemorrhagic dengue or shock
syndrome. In conclusion, this is the first confirmed outbreak of dengue
fever in Puerto Maldonado. Puerto Maldonado has changed from a
nonendemic to a hypoendemic dengue region of Peru.

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

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Dengue virus belongs to the family Flaviviridae and comprises four serotypes, designated as Den 1 - Den 4. The spectrum of illness ranging from mild fever to severe hemorrhagic fever and shock. The capture ELISA test is commonly used to detect IgM antibody as indication of recent infection but the definitive diagnosis of dengue is provided by the isolation/detection of virus in the patient’s acute blood or sera. The common cell culture method involves visualization by FA and most detection methods involve RT-PCR. Here we describe a novel virus identification method that combines cell culture and ELISA methodologies (ELISA-ICC). The ELISA-ICC utilizes 96 well plates in which tissue culture cells (C6/36 or Vero) have been seeded at 10^5 cells per well. Test specimen (sera) is added to a well, centrifuged and incubated for 1-14 days. The cells are fixed and the presence of virus is visualized using standard ELISA methodologies. The sensitivity of detection of eight dengue viruses (Den-1: West Pac 74, I.Q. 6'152, Den-2: S 16803, I.Q. 2913, Den-3: Ch53489, I.Q. 1728, Den-4: Y9008, I.Q. 1519) in C6/36, Vero and BHK cell lines have been examined. The range of virus detection in C6/36 cells after six days of incubation was 0.04 - 0.0002 PFU; Vero cells: 0.04 - 2 X 10^3 PFU and BHK cells required more than 1.2 X 10^4 PFU for virus detection. Fifteen acute sera (suspected to contain dengue virus) were tested for dengue virus by standard cell culture isolation methodology, RT-PCR, ELISA-ICC(C6/36) and ELISA-ICC(Vero). Standard cell culture isolation methodology identified dengue virus in 6/7 specimens. RT-PCR: 12/15; ELISA-ICC(C6/36): 12/15 and ELISA-ICC(Vero): 11/15. In conclusion, for dengue virus detection the ELISA-ICC (C6/36) method was superior to standard cell culture isolation methodology and equal to that of RT-PCR. Advantages of the ELISA-ICC (C6/36) method over RT-PCR include a lower cost per specimen and the method is less labor intensive.

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

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

CHARACTERIZATION OF DENGUE VIRUSES PREVALENT IN THAILAND

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Dengue virus is epidemic and endemic in virtually every country in the tropics. Dengue fever (DF) and dengue hemorrhagic fever (DHF) are serious illnesses in many tropical and subtropical countries. Dengue viral infection often causes viral hemorrhagic fever lead to death. A major question is why some of patients infected with dengue virus develop DHF while most of patients with symptomatic infections end up as DF. Many studies have been done in order to understand the pathogenesis of DHF. However, the mechanism of DHF remains unknown. Four types have been reported in dengue virus. Previous exposure to dengue infections increases the risk for severe diseases because antibody against dengue virus enhances the uptake of virus into macrophage. As a result of the enhancement of viral uptake, viral load is increased in patients. It possibly induces cytokine production drastically and causes DHF. Each type can be responsible for DHF. Several groups, however, reported biological differences between different types of dengue virus. All types have been prevalent in Thailand. However, DHF is able to occur in primary infection although it is rare. It has been reported that DEN-2 and DEN-4 can cause DHF only in secondary infection but DEN-1 and DEN-3 can cause DHF in primary infection in one fifth cases in Thailand. To understand the detail of the difference among these viruses, we characterized them by infection of C6/36 cells, HepG2 cells and I111 cells. In addition, we examined that host factors induced by infection with each dengue virus.

HLA MAY CONTROL VIRUS SEROTYPE SPECIFIC IMMUNITY IN DENGUE INFECTION

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Dengue fever (DF) is getting a serious public health problem in the tropics. In this study, we made an experimental design to identify the host gene(s) contributing to the development of Dengue Hemorrhagic Fever (DHF), or Dengue Shock Syndrome (DSS) in Vietnamese by hospital-based case-control study. The patients with DF, DHF or DSS were clinically diagnosed by WHO criteria, and their peripheral blood samples were collected at the Center for Preventive Medicine, Vinh Long Province (VL), and the Pediatric Hospital No.2, Ho Chi Minh City (NDH) in 2002 to 2005. The patients' age ranged between 10 months and 15 years. Two hundred age and sex matched control samples were collected in VL. The number of the patients with DF cases was 114, with DHF cases was 206, with DSS was 413 in total from two sites. HLA class I (HLA-A, B), class II (DRB1) and TNF-alpha promoter SNPs typing were performed.

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There was no significant difference in TNF-α promoter SNPs alleles and HLA-B. However, HLA-A*24 was significantly increased in DHF/DSS (P for trend = 0.0001) and HLA-DRB1*0901 was significantly decrease in severe patients (P for trend = 0.0001). These HLA-DRB1*0901 patients also showed resistance (P values 0.0007) to the most virulent serotype - dengue virus serotype 2. The DRB1*0901 allele might contribute to resistance and A*24 allele might contribute to susceptible to DSS in Vietnamese. These may have consequence for preventive strategies.

466

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

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

467

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

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

468

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

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Dengue is the most common arboviral disease in the world, with 50-100 million estimated cases of dengue fever and 250,000-500,000 cases of dengue hemorrhagic fever annually. In Puerto Rico, where dengue is endemic, the Department of Health and CDC maintain a laboratory-based surveillance system in which physicians are asked to submit serum samples from suspected dengue cases for diagnostic testing. From 1996 to 2004, the incidence of reported dengue in one rural municipality, Patillas, averaged three times that of the island. Enhanced dengue surveillance was instituted in Patillas in June 2005 to better estimate dengue incidence. In Patillas (population 20,300), one clinic provides care for 87% of residents. Clinic physicians were asked to complete a case investigation form and submit serum samples for dengue testing for patients meeting a strict clinical case definition for suspected dengue fever plus at least two symptoms (headache, eye pain, myalgia, arthralgia, rash, bleeding, thrombocytopenia, hemocoagulation, or shock). Laboratory-positive cases were defined as patients with dengue virus identified via PCR or viral isolation, seroconversion, or anti-dengue IgM positivity. From June 2005 through January 2006, the clinic reported 1096 suspected dengue cases. Of these, 136 (12%) were laboratory-positive including four hospitalized patients. The incidence of laboratory-positive dengue in this period was 6.7 per 1,000 population in Patillas, compared with 0.6 per 1,000 population for Puerto Rico and 0.1-1.6 per 1000 population for adjacent municipalities. In conclusion, the application of a strict clinical case definition in Patillas resulted in an incidence of laboratory-positive dengue infection over 11 times that of the island and at least four times that of adjacent municipalities. These findings suggest the magnitude of under-reporting of dengue fever in Puerto Rico and better illuminate the burden of dengue disease.
DENGLHE HEMORRHAGIC FEVER BY DENGUE 3 INFECTION. A RETROSPECTIVE SEROEPIDEMIOLOGIC STUDY IN HAVANA CITY, 2003

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

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

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

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

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Dengue is the most important arboviral disease in the world, and nowadays, dengue-3 virus constitutes a serious problem to the Brazilian public health system. Infections with dengue viruses result in different clinical syndromes, ranging from uncomplicated febrile illness to a potentially life-threatening syndrome, the dengue hemorrhagic fever/dengue shock syndrome. In this study, patients with suspected dengue-3 infections were evaluated and their clinical and laboratory findings compared to those present in other febrile illnesses. From February 2003 to June 2003, 80 patients with suspected dengue-3 disease were submitted to a complete physical examination, and blood samples were obtained for serological testing (MAC-ELISA), virus identification by reverse-transcription polymerase chain reaction (RT-PCR), complete blood count, and measurement of liver enzymes and creatine phosphokinase. All patients were adults with ages ranging from 12 to 56 years (mean: 30.7 years). Forty-five percent were male and 55% were females. Serology results showed that 47 (59%) were IgM-positive and 33 (41%) were IgM-negative. RT-PCR detected dengue-3 genome in 20 patient samples that had been collected from 2 to 10 days of disease onset (mean: 5.7 days). All of these patients were IgM-positive. Patients’ first medical evaluation ranged from 2 to 19 days after beginning of the symptoms (mean: 7.1 days). The most important findings and their frequency among IgM-positive and IgM-negative patients were as follows: thrombocytopenia (platelets <100,000/μL; 36% x 12%); AST and ALT levels 1.5-fold higher than the reference value (42.5% x 18.75% and 68% x 21%), respectively; hemocritiation (40.4% x 31.2%); leukocytes < 4000 cells/μL (42.5% x 3%); skin rash (85.1% x 21%); positive tourniquet test (36% x 15%); itching (55.3% x 12%); diarrhea (28.9% x 24.2%); fever (100% x 87.8%); retro-orbital pain (68% x 63.6%); headache (78.7% x 84.8%); and myalgia (89.3% x 90.9%). These results show that dengue-3 infections may be easily differentiated from other febrile illnesses, but attending physicians should not rely on “classic dengue symptoms”, such as fever, headache, and retro-orbital pain. On the other hand, leukopenia, skin rash, and itching are positively associated to dengue-3 infections.

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

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We report the findings of two years (2004-2005) of prospective study of dengue virus transmission and disease severity in a cohort of approximately 2010 children in 11 primary schools in northern Thailand. In 2004, 28 out of 33 confirmed (by Dengue/IE IgM/Ag EIA) acute dengue infections were positive for dengue viruses detected by nested PCR. Three out of four dengue serotypes were detected: 16 DEN-9, 9 DEN-2, and 3
DEN-3. In 2005, 15 out of 27 confirmed acute dengue infected children were positive for dengue virus detected by nested PCR. All four dengue serotypes were detected; 15 DEN-4, 2 DEN-1, 2 DEN-2, and 1 DEN-3. Dengue serotype 4 appeared to be the dominant circulated dengue strain in Northern Thailand in 2004 and 2005. The rate of severe dengue disease in 2004 and 2005 was similar, 6.33 and 5.27, respectively. In 2004, the ratio of inapparent dengue infection to symptomatic dengue infection was 2.7:1, but ranged greatly (0.9-9.0:1). Additional data from 2005 and 2006 will be presented along with analysis on the dengue incidences, the infecting dengue serotypes, and the rate and risks for symptomatic dengue disease.

473

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

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

474

EPIDEMIOLOGICAL ANALYSIS OF JAPANESE ENCEPHALITIS IN THAILAND, 2000-2005

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

475

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

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The current methodology for diagnosis of arboviral disease relies heavily on serological techniques such as the immunoglobulin (Ig) M antibody capture enzyme-linked immunosorbent assay (MAC-ELISA) as well as the indirect IgG ELISA, both of which require monoclonal antibodies (MAbs) specific for arboviral families. These tests are hindered by the lack of standardized calibrators and positive controls that react in assays designed for use with human sera for detection of clinically important arboviruses including dengue, West Nile, and Saint Louis encephalitis viruses. Our goal is to create chimeric antibody constructs expressing the variable regions of broadly cross-reactive murine MAbs and the constant regions of human IgM or IgG to produce positive calibrators of serologic (ELISA) assays for diagnosis of arboviral infection. The heavy and light chain variable regions from genus-specific murine MAbs for both alphaviruses (2A2C-3) and flaviviruses (686C-1) were amplified by reverse transcription polymerase chain reaction (RT-PCR) using degenerate primers annealing to conserved variable heavy (VH) and variable light (VL) gene leader sequences and constant (C) region specific primers. Multiple clones of each VH and VL gene fragment from 686C-1 and 2A2C-3 were sequenced and then modified by PCR to facilitate placement into immunoglobulin expression vectors containing genomic clones of either IgG or IgM C-region genes. A total of four separate plasmid constructs were created: pDH-IG (2A2C-3 variable region with human γ chain), pHIL-2M (2A2C-3 variable region with human μ chain), pDHIL-6G (686C-1 variable region with human γ chain), and pHIL-6M (686C-1 with human μ chain). These plasmids were used to transform Sp2/0-Ag14 cells by electroporation. Screening of transfecteds for expression and characterization of chimeric antibodies is ongoing.

476

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

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West Nile virus was isolated from the brain of a Blue Jay (Cyanocitta cristata) that died in November 2005 in Lafayette Parish, Louisiana. Because very few WNV isolates from Louisiana have been genetically characterized, we compared partial nucleotide sequences (premembrane, membrane and envelope genes) to previously published strains isolated in North America and Mexico. Pairwise alignment of the Lafayette strain with homologous regions of the prototype New York 1999 strain identified the
presence of ten divergent nucleotides. Most of the divergent nucleotides (80%) were pyrimidine-pyrimidine substitutions and only one, (U1442C) encoded for an amino acid substitution (Val-Ala at E159). A comparison of pairwise distances between all known IA isolates and NY99 demonstrates that the Lafayette 2005 isolate is the most divergent (0.5%), the overall mean distance is 0.283. Phylogenetic analyses suggest that this isolate is part of a recently described North American clade of dominant genotypes and differs from previously sequenced Louisiana isolates. The implications of these analyses and the need for additional studies are discussed.

**477**

**PREDICTING THE TRANSMISSION OF WEST NILE VIRUS**

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

**478**

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

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A small plaque variant (SP) of West Nile virus (WNV) was selected from an isolate (WT) made from the kidney of a dead crow in New York in 2000. We have characterized SP and WT viruses in vitro in African Green monkey kidney (Vero), chick embryo fibroblast (DF-1) and Aedes albopictus (C6/36) cells maintained at different temperatures, and in vivo in mosquitoes, birds and mice. The SP demonstrates delayed replication and lower virus titers than the WT in Vero and DF-1 cell culture at temperatures ranging from 34°C to 41°C. Significantly decreased growth of SP at 41°C in DF-1 suggests temperature sensitivity. No differences in growth rate or viral titers were noted in C6/36 cells between WT and SP at 26°C to 30°C. These results suggest higher incubation temperatures may be the cause of decreased replication in Vero and DF-1. Current studies are looking at lower temperatures of incubation for these cell lines, and higher temperatures for C6/36. Culex pipiens (L.) had lower infection and dissemination rates and a higher ID50, following preoral infection with SP. The mean virus titers of WNV SP in mosquito bodies are significantly lower than WT. Three days postinoculation, WNV SP also had a lower rate of virus replication and lower virus loads. Chicks demonstrated delayed peak viremia and much lower virus titers in peripheral blood following infection with WNV SP compared to WT. Adult house sparrows, natural hosts of WNV, were inoculated subcutaneously with 104 eID50 SP or WT. The viremic response of the SP infected birds was variable and the virus appeared to revert to WT. Further studies are underway to clarify pathogenesis in this host. C3H mice were inoculated SC in the footpad with 104 and 105 PFU WNV SP or WT. WNV SP had significantly lower morbidity and no mortality, and a lower viremia profile compared to WT. Virus was recovered with consistent high virus load from brains and footpads of all WT mice at the time of death in one experiment and on day 7 in a second experiment, and only from footpads of 7 of 8 infected mice following SP inoculation at 7 days PI with lower virus titers. No virus was recovered from brains of SP infected mice demonstrating that SP has lower neuroinvasiveness compared to WT. All data indicate that the SP variant is attenuated in vivo and in vitro. Further studies are being conducted in mice to evaluate neurovirulence.

**479**

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

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In an effort to understand the pathogenesis of West Nile disease in horses and the immune response to infection, a weanling, serologically naive male horse (ID1) and a weanling, serologically positive female horse (ID2) were challenged with 5x106 PFU WNV (NYC crow 99 strain) via intrathecal injection with complete physical and neurological evaluations performed for 28 days post inoculation (PI). Serum and plasma samples were collected before and after challenge for serological and virological evaluation. At D28, ID1 was euthanized to further characterize the model with a full gross and histopathological evaluation performed. The other horse, ID 2 was not euthanized for further immunological studies. Horse ID1 developed clinical signs of WN encephalitis at seven days post-infection including fever (38.4-40.1°C), depression, muscle fasciculations, ataxia, paresis, obtunded mentation, and tongue paresis. Recrudescence of signs occurred throughout the 28 day study period, clinical signs were completely resolved by D28 PI. In contrast, horse ID2 did not develop signs consistent with WNV encephalomyelitis. Mild depression occurred on D7 PI, but rectal temperature peaked at 38.7°C and no neurological deficits were noted throughout the 28 day study period. Viral cytopathic effects (CPE) were observed in Vero cell cultures incubated with EDTA plasma collected from ID1 on D1-4 PI. Evidence of viral RNA amplification in these samples was confirmed by Real Time RT-PCR. Antibody to WNV, IgM and neutralizing, was detected after infection, starting Day 10 through 28. Histopathological changes consistent with polioencephalomyelitis were observed in the brain and spinal of ID1. Neither viral CPE nor RNA was observed in Vero cell cultures and tissues, respectively. On day of infection (two months after initial screening) ID2 was negative for IgM (using MAC-ELISA), but neutralizing antibody (1:100) was detected on the serum collected before infection and throughout the study period. Cytokine analysis with Real Time PCR demonstrated the presence of IL-1β, IL-2, IL-6, IL-10 mRNA expression and limited expression of IL-4. Data obtained from this study is the first experimental evidence demonstrating immunity to rechallenge with virulent WNV in the horse and contributes to evidence that life-long immunity after flavivirus infection does occur in the horse.

**480**

**PERSEStANCE OF LYMPHATIC FilarIAsis FOLLOWING FIVE ROUNDS OF MASS DRUG ADMINISTRATION IN AN EGYPTIAN VILLAGE**

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Egypt is among the first countries to have successfully completed 5 rounds of mass drug administration (MDA) with DEC/Albendazole in
the context of a national lymphatic filariasis (LF) elimination program. The program was successful in most areas. However, surveillance data showed that microfilaremia (MF) prevalence rates remained above 1% in two sentinel sites (in 2 districts) after 5 rounds of MDA. All LF-endemic villages in these districts were treated with a 6th round of MDA. We now report data on MDA compliance and the effects of MDA on infection and transmission parameters in one of these problematic areas (Tania village, TR). We performed annual surveys (10% randomly selected households) and assessed MDA coverage rates, MF, by night blood smear, and filarial antigenemia by the ICT card test. Molecular xenomonitoring was performed with indoor resting mosquitoes pooled by household. We also assessed IgG4 antibodies to Bm14 by ELISA in primary schoolchildren (6-8 years of age) after rounds 3-5 as a marker for recent exposure to infection. Reported MDA coverage rates were 80.1%, 94.6%, 91%, 93.5% and 88.5% for MDA rounds 1-5, respectively. The MF prevalence rate decreased from 9.4% after MDA-1 to 1.5% after MDA-5 (84% reduction) whereas the antigen prevalence rate decreased from 25.1% to 8.6% (65.7% reduction). Antibody rates in grade-1 schoolchildren declined from 13.7% following MDA-3 to 4.2% after MDA-5, while mosquito infection rates declined from 6.1% (95% CI 5.85-10.47) after MDA-1 to 5.4% (3.98-7.07) after MDA-5. All of these results suggest that LF has been reduced but not eliminated in TR. This could be due to a high pre-MDA infection rate (estimated MF prevalence 15% by smear), systematic noncompliance or drug resistance (unlikely). Preliminary data following MDA-6 are not encouraging. Additional rounds of MDA would mostly treat uninfected people. Alternative options for managing areas refractory to MDA (DEC salt, vector control, targeted treatment) will be discussed.

481

HELMINTH INBREEDING AND THE DETECTION AND SPREAD OF DRUG RESISTANCE

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

482

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

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

483

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

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Delivery and coverage of mass drug administration (MDA) is vital to the effectiveness of the filariasis elimination effort. Equally important is determining when transmission has been reduced below sustainable levels. Thus, easy, effective and accurate monitoring of infection levels is critical to the elimination program. This study provides preliminary evidence of effective monitoring using mosquito infection as an indicator and aims to produce a comparison of measures across a longitudinal survey in Kirare Village, Tanzania, before and after a treatment program. The baseline pre-MDA mosquito infection rates were determined by the collection of mosquitoes during the period of Nov. 2003-Sept. 2004 by placing light traps in 50 randomly selected houses once per week. The dissection of 5,336 mosquitoes yielded a 1.4% rate of infectivity as determined by the presence of Wuchereria bancrofti L3 stage larvae. The post-MDA dissection results of 6,608 mosquitoes collected from Oct. 2004 through Sept. 2005 yielded a 0.4% infectivity rate. This 71% decrease in infectivity rate is significant with a z = 3.73, p<0.001.

In addition to monitoring by dissection, 1,514 mosquitoes collected from Jan.-July 2004 (pre-MDA) were tested in 135 pools using the molecular xenomonitoring (MX) PCR assay for W. bancrofti. The PCR assay estimates the mosquito infection rate by determining the presence of any stage of the parasite. Post-MDA collections of 2,374 mosquitoes collected from Jan-Oct. 2005 were tested by PCR in 211 pools. After only one round of MDA a decrease of 55% in the mosquito infection rate was found as assessed by MX, from a 15.6% probability of infection pre-MDA to a
7% probability of infection post-MDA (estimated by Poolscreen v.2.0.2). The reduction in infection rate as shown by PCR closely parallels the decrease in infectivity determined by dissection. In addition to monitoring the progress of the treatment program in Kirare, this study is extremely important in providing comparisons and evaluations of the monitoring tools available over the course of the entire treatment period.

(ACMOP Abstract)

484

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

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Lymphatic filariasis is a disfiguring and disabling disease that represents an important public health and socio-economic problem in Mali. As part of the international campaign to eradicate lymphatic filariasis, community-based mass chemotherapy with yearly administration of albendazole and ivermectin has been initiated in the district of Sikasso. The aim of this study was to evaluate the effects of this treatment on lymphatic filariasis transmission after three yearly treatments in the village of Kolekoba in Mali (population 3551). Following baseline entomological and parasitologic surveys in 2002, yearly mass treatment of the non-pregnant population (≥5 years old) was instituted. The coverage rate increased over the three year period from 67% in 2002 to 72.9% in 2005. The prevalence of parasitemia, as assessed by night-time thick smears, decreased significantly from 21.4% (n = 1141) in 2002 to 6.08% (n = 806) in 2005. The vector population was mainly composed of An. gambiae s.l. (94.62% n = 10622) followed by An. funestus. The mean biting rate declined each year from 605 in 2001 to 278.6 in 2005. Vector infection rates also declined over time with the highest infection rate of 4.2% in 2001 and the lowest (0.2%) in 2004. Finally, the EIR (Entomological Infective Inoculation Rate) decreased by 98.99% from 13.9% in 2001 to 0.14% in 2005. Although complete interruption of transmission has not occurred after three years of mass treatment in this area, significant decreases in both the prevalence of parasitemia and annual transmission potential have been observed. Increasing coverage rates and treatment of surrounding villages as the program expands will likely lead to improved results in the coming years.

485

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

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We studied and evaluated the evolution of Wuchereria bancrofti parasitemia in the population (near 1,400 people) of a filariasis-endemic area in Raiatea Island, French Polynesia, all along a 6-year period with annual DEC-ALB mass drug administration (MDA). Repeated surveys (pre-MDA evaluation in 2000, intermediate evaluation in 2005 and final evaluation in 2006) included tests for filariae circulating antigens, microfilariae and anti-filarial IgG in human blood as well as PCR-based poolscreening estimation of Aedes polynesiensis mosquito infection. Global results show that despite a MDA compliance rate between 79% and 98%, the evolution of the studied parameters is rather limited. Transmission indices represented by prevalence of microfilariae carriers and mean infection rate in mosquitoes only slightly decreased respectively from 7% to 4% and 2.5%[2-4] to 0.8% [0-3]. Besides, parasitemia index constituted by the prevalence of people positive in adult worm circulating antigens were shown to maintain above 10%. A detailed analysis of the area divided into 4 geographical sections is presented with the aim to scan such a situation and try to point out determinant factors.

486

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

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

487

ROLE OF GLUCOCORTICOIDs IN THE INNATE AND ACQUIRED IMMUNE RESPONSE OF MICE INFECTED WITH STRONGYLOIDES VENEZUELAENSIS

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

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last dose one hour before they were sacrificed; 3. The animals received the first dose on the 4th day after infection. Non-infected mice were used as control. On the 1st, 3rd, 5th, 7th, 14th, 21st and 37th day after infection, the animals were killed and the number of eosinophils in the blood, PCE and BALF as well as cytokines, antibodies and the number of larvae and worm parasites were quantified. Eosinophil number increased significantly in infected mice when compared with the control group. Deox significantly inhibited the number of eosinophils in the blood as well as migration to PCE and BALF in the three treatment schemes. Deox decreased the synthesis of TNF-α, IL-3, IL-4, IL-5, IL-10, IL-12, IFN-γ and IgG1, IgG2a and IgG2b antibodies. However, the number of larvae and recovered worm parasites were significantly increased. In conclusion, our data showed that Deox is a potent immunosuppressive drug which is capable of inhibiting eosinophils, cytokines and antibodies as well as helping parasitism in this experimental model.

488

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

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A parasitological survey was carried out in 15 localities in Bolivia, with altitudes ranging from 375 m to 2,125 m associated with great variation in climate and geology. Blood and faecal samples were obtained from 2,122 and 1,855 people respectively. Haematocrit was measured in the 2,122 blood samples and titration of Trypanosoma cruzi antibodies was carried out only in a target population (children under 3 and their mothers, i.e. 304 people). Faecal samples were analysed by MIF. Possible risk factors considered were (1) age, (2) gender, (3) family structure, (4) family size, (5) house size, (6) quality of house construction, (7) access to water, (8) place used for defecation, (9) economic indicators (e.g. quality of household appliances, number of cattle or poultry), and (10) length of residence in the village.

A highly significant positive correlation (P < 10−3) was found between altitude and either haematocrit or prevalence of Chagas disease. A highly significant negative correlation (P < 10−3) was found between altitude and prevalence of either anguillulosis or anguillulosis. A weaker but still significant (P < 5·10−4) negative correlation was observed between altitude and prevalence of trichocephalosis.

A highly significant positive correlation (P < 10−4) was found between presence of anguillulosis or anguillulosis and family size. A highly significant negative correlation (P < 10−3) was found between quality of house construction or house size and prevalence of either anguillulosis or anguillulosis. A weaker but still significant (P < 5·10−4) negative correlation was observed between age and prevalence of anguillulosis, between length of residence in the village and prevalence of either anguillulosis or anguillulosis and between poultry and prevalence of Chagas disease and amoebiasis. A weaker but still significant (P < 5·10−4) positive correlation was observed between access to water and prevalence of amoebiasis, presence of mammals near the house and prevalence of anguillulosis.

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

489

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

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Geohelminth infections are leading causes of chronic anemia and malnutrition worldwide, with a particularly high impact in impoverished rural communities. School-based deworming programs have been demonstrated to improve the growth and nutritional status of children living in areas endemic for geohelminths, including Ascaris lumbricoides, Trichostrongylus trichura, and hookworm. As part of a study aimed at defining the optimal deworming regimen in high risk populations, children from 10 primary schools in the Department of Izabal in eastern Guatemala were assigned to receive single dose albendazole treatment at either 6-month or 3-month intervals. Baseline enrollment data included analyses of 344 children aged 5-17 years. Of those, 229 stool samples were examined for the presence of helmint eggs via direct microscopy and the McMaster technique. Samples positive by either method were subsequently evaluated using the Kato-Katz technique in order to measure the intensity of infection (eggs per gm of feces). Of the 229 children examined, 69% were infected with at least one geohelminth: 30% for hookworm; 52% for Ascaris; and 38% for Trichuris. Among infected subjects, 59% had infection with 2 or more geohelminth species. Infections were more common in males than females (54% vs 46%), and more common in children living in rural than urban areas (36% vs. 44%), but neither difference was statistically significant. Anemia (blood hemoglobin level <11g/dL) was present in 4% of the 297 children studied, and occurred more commonly in geohelminth infected than uninfected subjects (13·1 g/dL vs 13·5 g/dL, p = 0·05), and in those living in close proximity to a lake (12·7 g/dL vs 13·3 g/dL, p = 0·0004). Based on infection intensity thresholds defined by the WHO, only 2% of hookworm infections were heavy, as were 4% of the Ascaris infections and 1% of the Trichuris infections. Increased intensity of hookworm infection was associated with a decrease in blood hemoglobin levels (p = 0·004), while no similar association was noted with Ascaris or Trichuris. These data confirm a high pre-treatment prevalence of geohelminth infections in school-aged children in eastern Guatemala. Future analysis of post-treatment growth and nutritional data will help define the optimal dosing regimen for children in this highly endemic area.

490

PECULIARITIES OF ANCYLOSTOMA CEYLUMANICUS L3 EXTRATION

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

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

491
HEMOZOIN DIFFERENTIALLY AFFECTS HIV-1 VIRAL REPLICATION ACCORDING TO THE SEQUENCE OF EXPOSURE

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Malaria and HIV-1 infections are responsible for 18% and 6%, respectively, of the mortality in African children under the age of five. Although co-endemicity of these pathogens is common in many tropical regions, molecular interactions between malaria and HIV-1 are largely undefined. Previous studies in our laboratory demonstrated that exposure to malarial pigment (β-hematin, synthetic hemozoin, sHz) increases simian immunodeficiency virus (SIV) production in rhesus macaques through a TNF-α-dependent mechanism. To further define interactions between malaria parasite products and immunodeficiency virus replication, we determined if the sequence of sHz exposure alters HIV-1 viral replication.

This was accomplished by alternating the initial exposure of PBMCs with either sHz or HIV-1. sHz treatment of PBMCs from healthy donors five days prior to HIV-1 infection (monotropic HIV-1NL4-3) increased HIV-1 p24 production, while sHz treatment following 24hrs of HIV-1 infection increased HIV-1 p24 concentrations in culture supernatants relative to controls. In an additional, an intracellular p24 assay was used to determine the amount of virus entering the cells. PBMCs were treated with sHz five days prior to a 3hr incubation with HIV-1NL4-3, then washed extensively to remove free virus, lysed, and the supernatants assayed for p24. These experiments revealed that treatment with sHz prior to HIV-1 exposure decreased HIV-1 particle entry. Cell viability was appropriately controlled for in all experimental paradigms with a tetrazolium salt-based assay.

Taken together, these studies demonstrate that the effect of sHz on HIV-1 viral replication is markedly different depending on the sequence of exposure to the two pathogens. Since our previous studies showed that sHz differentially regulates β-chemokine gene expression in cultured PBMCs, differential effects presented here may be related to chemokines competing for receptor binding with HIV-1. Future studies will determine the influence of chemokine/chemokine receptor interactions on HIV-1 viral replication during malaria/HIV-1 co-infection.

(ACMOP Abstract)

492
TOXICITY OF NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS (NRTI) IN MICE

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It is difficult to evaluate the toxicity of dual NRTI therapy in infants prenatally exposed to dual NRTI therapy. Thus, appropriate animal models are needed for testing and selecting safer combinations of antiretrovirals in order to prevent embryonic tissue damage. In this study, we exposed mice to NRTIs chronically, at doses calculated to cause limited toxic reactions in the dams but sufficient damage in the pups. We administered AZT and 3TC orally to 50 CO-1 pregnant mice divided into 3 groups according to treatment doses. We gave AZT and 3TC from E8.5 till birth. D1-2 by oral gavage, at a dose of 0 (control), 0.30/15 mg (treatment 1), 1.2/0.6 mg (treatment 2), and 4.8/2.4 mg (treatment 3). Tissues from liver, spleen and bone marrow were collected immediately after birth. The number and percentage of monocytes, lymphocytes and neutrophils were computed using standard techniques. We also evaluated the expression of CD3 and CD45 in the litter and spleen by immunohistochemical procedures. We used ANOVA to compare the differences in treatment responses. We considered a p-value < 0.05 as statistically significant. We found a no reverse correlation between antiretroviral dose and CD3/CD45 expression in the liver and spleen of mothers and pups. CD3 expression was also significantly reduced in brain tissues. The mean lymphocyte value for group 2 (34) and group (16.5) was significantly lower than for the control group (41.6), the mean macrophage value for group 2 (6) and group 3 (5.05) was significantly lower than for group 1 (9), and the mean neutrophil value for group 2 (6) and group 3 (5.05) was significantly lower than for group 1 (9). In conclusion, these results seem to suggest that AZT and 3TC induce lymphocyte toxicity. To determine the mechanisms of lymphocyte depletion, we need to conduct more studies including further observation of the mitochondria and related organelle changes. Long-term implications of large-scale perinatal therapy around the world are still poorly understood. These issues certainly underscore the need for animal model studies, which serve to select a safer perinatal antiretroviral drug regimen.

493
VALIDATION OF A LOW-COST SYSTEM FOR CD4+ T LYMPHOCYTE ENUMERATION IN RURAL BURKINA FASO

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Community-driven development (CDD), a model where resources are put in the hands of the beneficiary communities, has now been applied to the model of Association Driven HIV/AIDS Care and Treatment (ADCT) in several developing countries. Given the high prevalence of HIV disease, an accurate, low-cost method for CD4+ T lymphocyte monitoring to stage HIV infection and guide antiretroviral therapy is urgently needed. The EasyCD4 system (Guava Technologies, Inc.) uses microcapillary flow cytometry and has several advantages in comparison to standard technology. The study objective was to compare the performance of the EasyCD4 system to the FACSCount (Becton, Dickinson and Company) for community-based CD4+ monitoring of patients with HIV/AIDS in rural Sub-Saharan Africa. Blood samples from 98 HIV positive patients followed in a community HIV clinic in Ouahigouya, Burkina Faso were obtained for routine CD4 count monitoring. Each blood sample was divided into two aliquots, on which parallel CD4 count measurement was performed using the EasyCD4 and the FACSCount systems. Spearman rank correlation coefficient was calculated. Sensitivity, specificity and positive predictive value (PPV) for EasyCD4 <200/mm3 were determined compared to the standard FACSCount CD4 <200/mm3. Sensitivity, specificity and PPV were calculated using the breakpoint EasyCD4 <243.5 based on classification and regression trees. Mean CD4 count for the EasyCD4 and FACSCount were 313.75 and 303.47, respectively. Correlation coefficient (r) using the two methods was 0.92 (p<0.001). Median values using EasyCD4 were higher than those with the FACSCount (p=0.004). Using EasyCD4 >200, sensitivity is 71.8% (CI 55.1-85.3%), specificity is 94.9% (CI 89.5-98.9%), and PPV is 90.3% (CI 74.2-98%). Using EasyCD4 <243.5, sensitivity is 92.3% (CI 79.5-98.4%), specificity is 88.1% (CI 77.1-95.1%) and PPV is 83.7% (CI 69.3-93.2%). These results demonstrate that use of the EasyCD4 system is feasible for the enumeration of CD4+ T lymphocytes in rural Sub-Saharan Africa, particularly in the context of community-based HIV/AIDS healthcare. Additional studies will be required to determine the appropriate thresholds for initiation of antiretroviral therapy using low-cost diagnostic technology.

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494

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

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

495

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

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

496

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

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Enteric parasitic infections commonly cause diarrhea in patients with AIDS in resource-limited settings. They result in decreased quality of life and may compromise antiretroviral therapy (ART). Sensitive diagnostic tests to diagnose these potentially treatable infections are often unavailable in these settings. An on-going study at the GHESKIO Centers in Port au Prince, Haiti is examining patients with diarrhea at the initiation of ART. From October 2005 to date, 25 patients with diarrhea have been enrolled. 23 stool samples, collected at enrollment, are available for analysis. At GHESKIO, standard practices for stool evaluation includes a wet mount for ova and parasites and a modified Kinyoun acid-fast stain for coccal parasites. We used real-time PCR (qPCR) to detect the presence of Cryptosporidium DNA and to quantify oocysts from the stool samples. We also used a PCR assay that amplifies a conserved region of the small-subunit rRNA of all four major microsporidian pathogens, Encephalitozoon cuniculi, Encephalitozoon hellem, Enterocytozoon bieneusi, and Encephalitozoon intestinalis, followed by restriction endonuclease digestion by PstI to determine the prevalence of each species in the stool samples. 1023 patients (43%) had stool positive for Cryptosporidium DNA using qPCR. The median number of Cryptosporidium oocysts for patients was 700 per ml of stool. In contrast, only 323 (13%) were positive for Cryptosporidium by acid-fast staining. Restriction analysis indicated E. bieneusi DNA in 423 (17%) patients. No Microsporidia were identified by microscopy. The sensitivity of the current practice for the identification of Cryptosporidium infection in Haitian AIDS patients with diarrhea is 30%. In conclusion, molecular diagnostics for Cryptosporidium and E. bieneusi in AIDS patients with diarrhea improve rates of diagnosis of these potentially treatable infections. As nitazoxanide comes into wider use, accurate diagnosis of cryptosporidial infection may become more important. Albenazole can be used to treat Microsporidia infection. Continued efforts to develop rapid molecular diagnostic tests for resource-limited settings are essential.
ORAL MILTEFOSINE FOR CUTANEOUS LEISHMANIASIS IN THE DUTCH ELECTION SUPPORT FORCE IN NORTHERN AFGHANISTAN

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Preliminary results of treatment with oral miltefosine for cutaneous leishmaniasis in Dutch military personnel and one civilian who contracted cutaneous leishmaniasis in Northern Afghanistan in 2005 are presented. Initial therapy in 175 patients was with intralesional sodium stibogluconate, in some patients combined with cryotherapy. Thirty-three non-responders to topical therapy and one therapy-naïve patient were treated with miltefosine. The diagnosis was confirmed parasitologically in all 34 patients at initial presentation. Leishmania major was demonstrated in all PCR-positive cases. Treatment with miltefosine was 50 mg 3 times a day for 28 days. Patients' body weight ranged from 70 to 112 kg. Response to treatment was assessed clinically and by parasitological methods of PCR and Quantitative Nucleic Acid Based Amplification (QTNASA). Assessment was done at the end of treatment, at 6 and 24 weeks thereafter. Nausea was reported by 21 (61.8%) patients, vomiting by 14 (41.2%) and fatigue by 10 (29.4%). Five patients (15.3%) spontaneously reported a transient reduction of seminal fluid volume during treatment. Two of these patients also reported transient scrotal pain and epididymal swelling. Mild elevation of transaminases and creatinine was seen in 6 (17.6%) and 1 (2.9%) respectively. All abnormalities were reversible. Adverse effects did not lead to non-compliance or absenteeism. 29/34 (85.3%) patients presented with lymphonodular involvement during treatment. This lymphonodular reaction was also seen in untreated and topically treated L. major infections. Follow-up is ongoing. Directly following treatment, 19/34 (55.9%) and 1/53 (3.5%) showed clinical and parasitological recovery by PCR, respectively. After 6 weeks 23/31 (74.2%) and 21/29 (72.4%) showed clinical and parasitological recovery by PCR, respectively. Three patients (8.8%) have shown recrudescence requiring additional treatment. In conclusion, miltefosine is a reasonably well tolerated and effective treatment for cutaneous leishmaniasis due to L. major in Northern Afghanistan.

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

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Canine visceral leishmaniasis is caused by a protozoal parasite of the specie Leishmania (L.) chagasi, endemic for humans and dogs in many regions of Brazil. The aim of this study was to investigate the parasitological and histological pattern of the skin lesions from 34 dogs grouped in symptomatic (n = 9), olsigomatic (n = 17) and asymptomatic (n = 8) according to the clinical signs of the disease. All dogs were submitted to direct parasitological examination by direct microscopic visualization of promastigotes in stained tissue smears or in paraffin embedded tissues (histopathology) and serological examinations (ELISA and IFAT). The dermal inflammatory pattern and the cell population were evaluated histologically on HE-stained sections. The parasite load was determined by 100 microscopic field examination (magnification of 1000x) and it was graded from + to ++++. In the asymptomatic group: 4 dogs (50%) were negatives and 4 were positives showing grades ranging from + (12%), ++ (25%) to +++ (12%). The oligosymptomatic group had 2 (12%) negatives and 15 infected with grade from + (29%), ++ (35%), +++ (12%) to ++++ (12%). The asymptomatic group had only positive dogs with the grade ranging from + (22%), ++ (11%) to +++ (67%).

The majority of the parasites were loaded inside the macrophages near to the epidermis or in the mid-derms around the small vessels, hair follicles and sebaceous glands. The most severe lesions and the greatest parasite load were seen in the symptomatic dogs. The most of tissues showed areas with chronic inflammatory leesion characterized by the presence of large numbers of macrophages, lymphocytes and plasma cells. 92% of skin with microscopic lesion and only 40% without lesion were positive with amastigotes. The serological tests showed that 62% of asymptomatic dogs, 18% of oligosymptomatic and 12% of symptomatic were negatives and there was no agreement between serological and parasitological examination in 5% of the cases.

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

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There is no gold-standard test for the diagnosis of latent Chagas disease. Multiple antigen binding assay (MABA) is attractive because it can be simple, rapid and specific. In the MABA format, different antigens can be used on the same strip, conserving serum. The objective of this work was to assess a MABA test based on trypanosomagote excreted/secreted antigen (TESA Tulahuen) and a 55 kDa immunofinity purified TESA protein as a confirmatory test for latent Chagas disease. A total of 66 individuals were recruited: 74 female (52%) and 32 male (48%) and mean age 23 years (standard deviation: 14.17), in a rural community with heavy domestic triatome insect infestations rates. Serum samples were analysed by an EIA using fixed-epimastigote antigen that were grown in axenic culture (LT medium) and the novel MABA test. The EIA test identified 7 positive sera (detection rate 11%) only 6 out of which were confirmed by MABA (detection rate 9%). These data suggested that MABA test could be used either as a second "routine" test (e.g: for blood banking screening) or as a confirmatory assay for the diagnosis of latent Chagas disease. The high detection rate for anti- Trypanosoma cruzi antibodies found suggest a clear lack of an adequate control of Chagas disease in this region of Venezuela.
500

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

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The incidence of seropositive blood donors in North America has increased with population migration and increased surveillance. The USA, considered non-endemic for Trypanosoma cruzi, could therefore be at risk to exposure to disease transmission through blood or organ donations. Current tests show variable reactivity, especially with Central American sera. Here we describe the development of a lateral flow immunosassay for rapid detection of Tcruzi infection that has a strong correlation to the RIPA “gold standard” and is applicable for use in geographically diverse samples particularly from Central America. Such a test would have utility in small blood banks for pre-screening donors as well as in cardiac transplantation evaluation. T. cruzi consensus and/or RIPA positive sera were evaluated in EIA. These included commercial panels from Boston Biomedica Inc (BBI) (n=14), and Tergagenex (n=21). Both sources represented samples from different Central and South American countries. Other sources included a blood donor panel mainly from Costa Rica, Nicaragua, Honduras and Guatemala (n=205) as well as RIPA positive sera from the American Red Cross (ARC) (n=44). Sera were tested with the multiple recombinant Tcf and Peptides 1.30,36,SAFA and 1.1,1.2 and 1.3. Of the Central American blood donor panel tested only with Tcf; 31 were positive. 29 were positive by Organon EIA and 172 negative by both tests. 2 were Organon EIA positive only. In addition, all BBI samples were positive and 7/21 Tergagenex samples and 6/44 ARC samples were low positive or negative. This indicated the need for additional antigens. To complement Tcf reactivity in Central American sera we identified a promising combination of the tested antigens and constructed a single recombinant protein that exhibited full reactivity of the complementary antigens and enhanced clinical sensitivity in Central American sera. Data on its evaluation with RIPA confirmed positive sera will be presented. In addition, antigens identified from serological expression cloning with RIPA positive, Tcf negative sera are being evaluated for potential use in the rapid assay as necessary.

(ACMIP Abstract)

501

PREVALENCE OF TRICHOMONIASIS IN IMO STATE, NIGERIA

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

502

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

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

503

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

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Vertical transmission of Trypanosoma cruzi is well documented. What is less certain is whether all strains exhibit similar transmission patterns. This is especially of interest in North American versus South American strains of the parasite since in North America T. cruzi has evolved with placental mammals as the principal sylvatic reservoir hosts, while in South America marsupial species have dominated. Female BALB/c and outbred mice were infected with either the Brazil strain (BS), a Type I South American isolate, or a Type IIa isolate (SC) from North America. Breeding pairs were established and monitored for reproductive success. Pups were sacrificed at two weeks and tissues processed for diagnostic PCR using the TCZ primer set. BALB/c mice infected with the BS of T. cruzi failed to generate any offspring. Those infected with the SC isolate produced offspring in comparable numbers to those of uninfected control females. Of those pups born to Li infected female mice, 74% were PCR positive for T. cruzi. In the outbred mice 67% of the pups born to SC isolate infected females were positive (n=142), while from BS infected females only 33% of the
pups (n=132) were infected. This data suggests that the Type Ila SC isolate is significantly more disposed to vertical transmission than the Type I IBS. This is consistent with the co-evolution of this strain in placental mammals and possibly represents an increased reliance on this mechanism for transmission.

504

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

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

505

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

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Introduction of Trypanosoma cruzi, the causative agent of Chagas disease, into its mammalian host initiates a complex immune response capable of controlling parasitemia in the acute phase but unable to completely clear the infection, resulting in a life-long chronic infection. While studies have shown the CD8+ T cell response is essential for control of parasites during both the acute and chronic phases of infection, less is known about the generation and maintenance of the memory CD8+ T cell response during T. cruzi infection. In this study we used the C57Bl/6 mouse model of T. cruzi infection to determine the parasite-specific memory CD8+ T cell response to IL-7 and IL-15, two cytokines critical for the survival of memory CD8+ T cells after pathogen clearance in viral and bacterial infection models. To identify parasite-specific CD8+ T cells, we used MHC class I tetramers containing the immunodominant trans-sialidase peptide, TSK829. The majority of TSK829-specific CD8+ T cells from mice with chronic T. cruzi infections did not express the high affinity IL-7Rα chain (CD127), the receptor required for responsiveness to IL-7. TSK829-specific CD8+ T cells also failed to proliferate in the presence of IL-7 and IL-15 in vitro, consistent with the observed response of T cells in models of chronic viral infection. We also used an experimental model of T. cruzi infection in which the drug benznidazole was used to cure C57Bl/6 mice infected with T. cruzi. Parasitological cure resulted in a change in phenotype of the majority of TSK820-specific CD8+ T cells to high expression of CD127. These results suggest that T. cruzi-specific CD8+ T cells in infected mice fail to develop bona fide central memory characteristics as a result of persistent antigen exposure during T. cruzi infection. However in the absence of parasites, a CD127-expressing central memory CD8+ T cell population emerges.

506

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

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We have established previously that upon infection with Leishmania (V) panamensis, neutrophils from male hamsters are associated with higher parasite burdens and more severe lesions than resistant female hamsters. In this work we evaluated the role of neutrophils in male hamsters inoculated intradermally in the snout with 106 stationary phase promastigotes. Following L. panamensis inoculation there were distinct peaks in the number of intraepithelial neutrophils at 6 h and 21-25 days p.i. (subacute phase). Depletion of neutrophils before the first peak (6 h) using either polyclonal antibodies or cyclophosphamide resulted in a 5.2 and 6.4 fold increase, respectively, in the percentage of infected macrophages infiltrating the inoculation site at 6 hours p.i. compared with controls (p<0.001). On the contrary, systematic administration of IFN-γ (r-IFN-γ; Met-Leu-Ph) that increased the number of neutrophils during the first peak, resulted in a 2.4 and 8.4 fold decrease in parasitized macrophages at the inoculation site at 6 h and 19 days p.i., respectively (p<0.001). Depletion of neutrophils at the time of the second peak was associated with diminution of both the lesions (p<0.001) and proportion of amastigote-laden macrophages (6.5 to 7.9-fold reduction; P<0.001) at 22 and 45 days p.i. In contrast, at the latter time point (45 days p.i.), hamsters with increased numbers of neutrophils showed larger lesions (p<0.001) and higher amastigote densities (1.8 fold increase, p<0.001) than controls. These findings indicate that in this model of chronic cutaneous leishmaniasis, neutrophils participate in the initial control of Leishmania, but subsequently promote macrophage infection and lesion development.

507

ADJUVANT EFFECT OF GARLIC EXTRACT WITH A DNA VACCINE AGAINST LEISHMANIA

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Leishmaniasis is a group of diseases caused by protozoa of diverse species of the genus Leishmania. A DNA vaccine encoding Ni36 partially protected BALB/c mice against L. mexicana and L. chagasi infection. Garlic extract was also found to be a potent immunomodulator for the therapy of L. major and L. mexicana infection. We thus tested here the adjuvant effect of garlic extract with the NH36 DNA vaccine against L. mexicana and L. chagasi infection. Garlic bulbs were dried, extracted with water and the solution was filtered. The DNA vaccine VR1012-NH36 was produced from a culture of E. coli. BALB/c mice were vaccinated with VR1012-NH36.
DNA with or without dry garlic as adjuvant, and infected with L. mexicana or L. chagasi. The progress of the infection was evaluated by the size of the esions measured ver the rotary or parasitic load by Units of leishman donovan (LDU). The type of immune response was determined by phenotyping T lymphocytes by flow cytometry, measuring the production of IFNγ, delayed type hypersensitivity (DTH), and antibody levels. Mice vaccinated with VR1012-NH36 plus garlic extract and infected by L. mexicana developed lesions larger than those that received the vaccine alone. The two doses of garlic extract tested together with VR1012-NH36 against L. chagasi infection did not improve protection, and while garlic alone potentiated the immune response, it did not affect parasite load. Thus, this study confirms the efficacy of the DNA vaccine alone, against both Leishmania species, and indicates that garlic extract has no adjuvant effect with the NH36 DNA vaccine.

(ACM/CIP Abstract)

508
IDENTIFICATION OF NEW LEISHMANIA VACCINE CANDIDATES BY BIOINFORMATIC ANALYSIS OF LEISHMANIA MAJOR GENOME AND IN VIVO VALIDATION

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Leishmaniasis is a worldwide disease with an estimated 12 million infected people and the population at risk is 350 million. The recent completion of sequencing of Leishmania major genome together with bioinformatic tools open opportunities for the rational development of vaccines and identification of antigens. The objective of this work was that to identify new vaccine candidates by bioinformatic analysis of the L. major major gene and validate in vivo their immunogenicity. 8,272 translated sequences from the annotated L. major Friedlin genome were analyzed with RANKPEP epitope prediction algorithm to predict MHC class I mouse epitopes (H2-Kd and H2-Dd alleles). A total of 627 genes containing epitopes with MHC binding scores >85% were reanalyzed with additional epitope prediction programs to establish consensus predictions, using 5 distinct algorithms for H2-Dd and 8 for H2-Kd. 79 genes with top consensus predictions (4/5 or 8/8 for H2-Dd and H2-Kd, respectively) were further analyzed to identify sequences conserved in other trypanosomatid and eliminate sequences conserved in human and mouse genomes. Most of these genes encode for hypothetical proteins (64/79), and only 15/79 have a putative function. Interestingly, only 67/9 seem to be membrane-associated or surface proteins, and the remaining are intracellular. 20 of the top scoring genes were selected for validation in vivo. Transcriptionally active PCR products are being prepared for direct immunization of BALB/c mice and evaluation of their immunogenicity by flow cytometry and cytokine assays. We expect to be able to identify new potential vaccine antigens with this strategy.

(ACM/CIP Abstract)

509
RELATIONSHIP OF POLYMORPHISMS IN PLASMODIUM VIVAX DUFFY BINDING PROTEIN AND P. VIVAX PARASITEMIA AND SUSCEPTIBILITY TO RE-INFECTIONS IN A PROSPECTIVE COHORT STUDY OF PAPUA NEW GUINEAN CHILDREN

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

510
P. FALCIPARUM FCR3ΔVAR2CSA MUTANTS- A NOVEL TOOL TO EVALUATE PARASITE LIGANDS INVOLVED IN PLACENTAL MALARIA

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In high transmission regions, protective clinical immunity to Plasmodium falciparum develops during early years of life, limiting serious complications of malaria to young children. Pregnant women are an exception and are especially susceptible to severe P. falciparum infections resulting mainly from the massive adhesion of Plasmodium falciparum infected erythrocytes (IE) to placent al syncytiotrophoblasts. Chondroitin sulfate A (CSA) present in the placental intervillous blood spaces has been described as the common receptor involved in the massive sequestration of IE to the placenta. However, it is controversial if other receptors are involved in placental sequestration. We previously reported that disruption of a particular member of the var gene family (var2csa) results in the inability of the FCR3Δvar2csa parasites to recover binding to CSA. In this study we used our FCR3Δvar2csa mutant to investigate if the parasite genome encodes for adhesion molecules that bind to non-CSA placent al receptors. Experimental evidence suggested that placental parasites could adhere to hyaluronic acid (HA). Multiple rounds of selection of the FCR3Δvar2csa IE on bovine HA did not result in the selection of HA binding IE, whereas FCR3 wild-type parasites selected on bovine HA resulted in the selection of IE with the CSA- and HA-binding phenotypes. In order to verify the HA binding specificity, different sources of HA were used and revealed that the observed binding results from CSA contamination. Multiple passages on the placental derived B6Wo cell line resulted in the emergence of parasites able to cytoadhere in a CSA-independent manner. We are currently investigating the parasite ligand and its role in pregnancy-associated malaria.
150

511

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

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

512

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

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

513

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

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Sickle hemoglobin (HbS) results from a single point mutation (glutamic acid to valine) in the β subunit of hemoglobin. A high frequency of the HbS variant is likely maintained in sub-Saharan Africa because heterozygous carriers (HbAS) have increased resistance to Plasmodium falciparum malaria. We have previously shown that HbAS protects against childhood severe malarial anemia (SMA: Hb<6.0 g/dL), but not high-density parasitemia (HDP: 10,000 parasites/μL or greater) in a P falciparum holoendemic area of western Kenya. Our investigations in this region further revealed that elevated numbers of pigment (hemozoin, HZ)-containing monocytes (PCM) are significantly associated with enhanced development of SMA. To extend these findings, we determined that HbAS was associated with protection against SMA by decreasing HZ acquisition by monocytes and neutrophils in children with acute malaria (n=618, aged <3 yrs). Multivariate logistic regression controlling for age, gender, and HIV-1 status, revealed that children with HbAS had reduced numbers of PCM compared to children with normal Hb alleles (HbAA) [0.51 (0.32-0.83); p<0.01]. Stratification of children into low (10% or less) and high (greater than 10%) numbers of PCM further demonstrated that HbAS protected against both low [0.56 (0.24-0.94); p<0.05] and high [0.09 (0.01-0.70); p<0.05] levels of PCM. However, no significant relationships were found between Hb genotypes and the number of pigment-containing neutrophils. Taken together, results here show that HbAS-mediated protection against SMA may be due, at least in part, to decreased monocyte-acquisition of HZ. Since accumulation of HZ occurs during the late stages of the Plasmodium life cycle (i.e., late trophozoites and schizonts), we hypothesize that carriage of the Hbs variant may reduce HZ deposition by promoting immune recognition and clearance of parasites during early stages of the erythrocytic cycle (i.e., rings and early trophozoites) where HZ synthesis is minimal.

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