Beninese pregnant women, this study aims to evaluate the clinical burden of malaria in a highly endemic area. An ongoing prospective cohort of 1039 women followed monthly from their first antenatal visit (ANV) until delivery is conducted in three rural dispensaries since August 2008 in Benin. 570 women seen at ANVs, unscheduled visits and at delivery were analysed for the presence of symptoms. We used a multivariate logistic regression to determine the association between symptoms and malaria infection assessed by a positive rapid diagnostic test (RDT). During routine ANVs, headache was the only symptom associated with a higher risk of malaria (aOR=2.6; p<0.001) and was reported by 35% of infected women. On the occasion of unscheduled visits, fever (aOR= 4.1; p<0.001), headache (aOR= 2.1; p=0.01) and shivering (aOR= 3.2; p<0.001) were significantly associated with a malaria infection and 82% of infected women presented at least one of these symptoms. We found an increasing proportion of positive RDTs in late pregnancy more than one month after the last intermittent preventive treatment dose (IPTp); moreover malaria infections during unscheduled visits occurred long after the last IPTp intake. In conclusion, the majority of pregnant women were symptomless during routine visits when infected with malaria in an endemic stable area. Only, during unscheduled visits a significant proportion of infected women were symptomatic. The prevention of malaria in pregnancy could be improved by using systematic RDTs to identify infected women consulting during non routine visits. The design of IPTp could also be optimized by reassessing the number of doses and time of administration of SP.

155

NATIONWIDE PREVALENCE OF MALARIA IN CAMBODIA IN 2007: COMPARISON OF MICROSCOPY AND PCR

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In order to assess the current status of malaria in Cambodia and to compare it with the situation found in 2004, a nationwide malaria survey was conducted in November-December 2007, at the end of the rainy season, the time of peak malaria transmission. This was a stratified, multi-stage, cluster sampling survey. The country was divided into three domains based on expected malaria prevalence. The domain that included the provinces immediately around Phnom Penh was not surveyed, due to the very low prevalence found in previous surveys. The remaining provinces were divided into domains 1 and 2. Within each domain 38 clusters (villages) were selected; the clusters were stratified according to risk zones based on the distance from the village to the nearest forest (<250 m, 251-1000 m, 1-2 km, 2-5 km). Within each cluster 40 households were sampled, and from each household, 4 individuals provided malaria smears and filter paper blood spots for PCR-based diagnosis using the mitochondrial cytochrome b gene as a target. Based on microscopy, the overall estimated malaria prevalence and prevalences of *P. falciparum* and *P. vivax* infection in the sampled domains were 2.9% (95% CI, 1.8-4.6%), 1.6% (0.9-2.7%), and 0.9% (0.6-1.6%) respectively. The corresponding prevalences found in 2004 were 4.4% (2.8-6.8%), 2.9% (1.7-5.1%), and 1.3% (0.8-2.1%); this decline in prevalence, while appreciable, was not statistically significant. In order to determine the extent to which microscopy might underestimate the malaria prevalence, we performed PCR on 7707 samples; in these samples the malaria prevalences estimated by microscopy and PCR were 2.8% and 6.9%, respectively; 289 of 7162 microscopy negative samples (4.0%) were positive by PCR. The high prevalence of infection undetected by microscopy suggests that prevalence surveys based only on microscopy may significantly underestimate malaria prevalence. If these sub-microscopic infections contribute to transmission, then mass screening and treatment based on microscopy alone may miss a significant reservoir of infection.

156

SPATIO-TEMPORAL DISTRIBUTION OF MALARIA IN HAINAN PROVINCE, CHINA

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Hainan Province is one of the regions of the highest malaria incidence in China. Our study analyzed the distribution of malaria and the change of main epidemic areas from 1995 to 2008, to provide basis for the prevention and control of malaria in Hainan Province. The study was based on the data of each county/city between 1995 and 2008. Records of malaria cases were obtained from Hainan Center for Disease Control and Prevention and demographic data from Hainan statistical yearbook. Cluster analysis of time-space scanning was performed with the maximum cluster size of 25% of the population used SatScan 8.0. The temporal cluster analysis of 1995-2008 showed that 2003-2004 was the most likely cluster (*RR*=1.86, *P*=0.001). The space-time cluster analysis of 1995-2008 showed 7 counties/cities (San Ya, Bao Ting, Le Dong, Wu Zhishan, Ling Shui, Bai Sha and Qiong Zhong) in 2003-2004 was the most likely cluster (*Incidence*=2671.0/100,000, *RR*=4.97, *P*=0.001). The space-time cluster analysis of 1995-2002 showed 5 counties/cities (Bao Ting, San Ya, Wu Zhishan, Ling Shui and Qiong Zhong) in 1997-1998 was the most likely cluster (*Incidence*=1852.8/100,000, *RR*=4.49, *P*=0.001) and 3 counties/cities (Chang Jiang, Dong Fang and Bai Sha) in 2001-2002 the secondary one (*Incidence*=1258.6/100,000, *RR*=3.25, *P*=0.001). The space-time cluster analysis of 2005-2008 showed 5 counties/cities (Ling Shui, Bao Ting, Wan Ning, Qiong Zhong and Wu Zhishan) in 2005 was the most likely cluster (*Incidence*=1193.7/100,000, *RR*=4.55, *P*=0.001) and 4 counties/cities (Dong Fang, Chang Jiang Le Dong and Bai Sha) in 2006 the secondary one (*Incidence*=1038.9/100,000, *RR*=3.30, *P*=0.001). In conclusion, during 1995-2008, malaria incidence reached its peak in 2003-2004 and the southern Hainan Province was the main epidemic area. Although the average incidence decreased, the main epidemic area was expanded to the southeastern and southwestern Hainan Province gradually. Hence, future public health planning and resource allocation in Hainan Province should be focused on these areas.

157

INSIGHT INTO ANTIGENIC DIVERSITY OF VAR2CSA-DBL5ε DOMAIN FROM MULTIPLE *PLASMODIUM FALCIPARUM* PLACENTAL ISOLATES

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High levels of anti-VAR2CSA antibodies levels are associated with protection against pregnancy-associated malaria. VAR2CSA contains molecular signatures associated with parity in one of its domain, and variants preferentially infecting primigravidae are thought to be the most virulent. Therefore it is critical to identify sequence characteristics of this molecule that can interfere with immune response. Highly conserved domains of VAR2CSA such as DBL5ε are likely to contain conserved epitopes, and therefore constitute attractive targets for vaccine development. Sequences of the VAR2CSA-DBL5ε domain obtained from cDNA of 40 placental isolates were analysed by experimental and in silico tools. Competition ELISA assays on two DBL5ε variants, using women plasma samples from two different areas and mice specific antisera, indicated that DBL5ε possess conserved and cross-reactive B cell epitopes. Peptide ELISA identified conserved areas that are recognised by naturally acquired antibodies. Specific antibodies against these peptides

labelled the native proteins on the surface of placental parasites. Despite high sequence homology, both VAR2CSA DBL5ε recombinant proteins displayed different recognition patterns by plasma from malaria-exposed women, and their ability to bind proteoglycans. Sequence analyses showed that, like the previously characterised VAR2CSA DBL3X domain, DBL5ε also contains motifs that discriminate parasites according to donor's parity. In conclusion, this study provides insights into conserved and exposed B cell epitopes in DBL5ε that can act as potential mediator for cross reactivity. The importance of sequence variation in VAR2CSA as a critical challenge for vaccine development is highlighted. As the final conformation of the entire VAR2CSA molecule seems to be essential to its functionality, identification of sequence variation sites in distinct locations within VAR2CSA that affect its antigenic and/or binding properties is of major interest in the effort of developing an efficient VAR2CSA-based vaccine. Motifs associated to parasite segregation according to parity are among these critical issues.

158

SITE CHARACTERIZATION FOR A MALARIA VACCINE TRIAL IN THE SAPONÉ HEALTH DISTRICT IN BURKINA FASO: SEASONAL PREVALENCE OF MAIN PARASITES INFESTATION

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Populations living in areas where future malaria vaccine trials may be conducted must be characterized not only with respect to the parameters that will be used to establish safety, but also with respect to conditions that may modify the immune response to candidate malaria vaccines. Studies conducted in Africa and Asia indicates that helminths can influence the acquisition of immunity against *Plasmodium* by driving the immune responses towards the production of the non-cytophilic subclasses. The aim of this study was to estimate the prevalence of various parasitic infections according to season in potential participants to malaria candidate vaccine trials in Burkina Faso. We conducted 2 community-based cross sectional surveys in volunteers aged 2 to 45 years in the Sapone health district. Survey 1 was performed during the rainy season, and the second at the dry season. During each survey, clinical examination has been performed and blood samples have been taken for malaria and *Wuchereria bancrofti* diagnosis. Stools and urine were also collected for determination of helminthes and *Schistosoma hematobium*. The diagnosis of intestinal helminthes was done by Kato-Katz thick smear examination technique. The mean age of the volunteers was similar during the 2 surveys (p=0.44). From 1587 stools samples analyzed, 132 (8.3%) had helminth or other intestinal infections. The prevalences were higher at the rainy season as compared to the dry season. The main helminth infections were *Ankylostoma duodenale* (5.9% vs 2.1%; P<0.00), *Ascaris lumbricoides* (1.7% vs 0%; P<0.00), *Trichuris trichiuria* (0.8% vs 0%; P<0.00). Others intestinal parasites were *Hymenolepis nana* and *Taenia* sp. (4.5% vs 2.1%; P<0.00). The seroprevalence of *W. bancrofti* was 11.0% (12.8% vs 9.4%, P=0.03). *S. hematobium* infection was present in 2.3% (1.7% vs 2.9%, P=0.13) of the study population. According to age group the prevalence of malaria infection was higher at the rainy season (< 5years: 68.9%; ≥ 5years: 54.8%) as compared to the dry season (< 5years: 57.8%; ≥ 5years: 46.3%). In conclusion, these data show diversity and intensity of parasitic infections in Saponé health district area according to malaria transmission season. The trends of helminths infections and malaria infection coincide and are both high during the malaria high transmission season. This should be considered when designing future malaria vaccine trial.

159

IMMUNOLOGICAL EFFICACY OF VACCINE-INDUCED ANTIGEN-SPECIFIC CD8+ T CELLS AGAINST *PLASMODIUM YOELII* BLOOD STAGE INFECTION

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There is a consensus that CD8+ T cells are critical for conferring hosts' protective immunity against the malarial liver stage; however on the contrary, the lack of MHC molecule on red blood cells has questioned their protective roles against its blood stage infection. This skepticism was supported by an observation that the depletion of CD8+ T cells during the malarial blood stage infection did not affect its natural course and outcome. However, since the CD8+ T cell-mediated vaccine strategy has presented new development in recent years, it is worth elucidating the immunological efficacy of the active induction of antigen-specific CD8+ T cells, particularly the prime-boost vaccination strategy which is the most effective vaccination protocol for the induction of maximal number of antigen-specific CD8+ T cells. To address the question whether the actively induced CD8+ T cells in maximal number are capable for conferring hosts' protective immunity against the malarial blood stage, we have established an experimental system by generating a genetically engineered *Plasmodium yoelii* which expresses a *Trypanosoma cruzi* antigen-derived, H-2Kb-restricted-CD8+ T cell epitope, ANYNFTLV. Expression of the epitope by the transgenic parasites was confirmed by the detection of ANYNFTLV-specific CD8+ T cells in mice, either which were immunized with adjuvant-emulsified parasitized red blood cells or which were cured by the injection of chloroquine after the infection with transgenic parasites. We have then tested the immunological efficacy of the prime-boost recombinant virus vector vaccination, the multiple passive transfers of ANYNFTLV-specific CD8+ T cell line and the combination of both against the challenge infection with the ANYNFTLV-expressing transgenic parasites. The critical roles of CD8+ T cells during the malarial blood stage infection and their background immunological mechanisms will be presented and discussed.

160

HETEROLOGOUS PRIME-BOOST VACCINATION WITH ADCH63 AND MVA EXPRESSING MSP1 CAN INDUCE PROTECTIVE EFFICACY AGAINST SPOROZOITE CHALLENGE IN VOLUNTEERS

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Viral vectored vaccines encoding blood-stage malaria antigens can stimulate potent cellular and humoral immune responses in mice and rhesus macaques and induce protective efficacy in rodent malaria models. We sought to test the safety, immunogenicity and efficacy of this approach in a Phase I/IIa clinical trial using the simian adenovirus 63 (AdCh63) and the poxvirus MVA encoding a novel insert including conserved blocks of sequence and both alleles of the 42kDa C-terminus of the blood-stage malaria antigen MSP1. In a Phase I dose escalation