Advances in *Plasmodium vivax* Malaria Research

Poster Sessions

Tuesday, May 28, 2013
6:35pm – 8:00pm

Wednesday, May 29, 2013
1:00pm – 2:30pm

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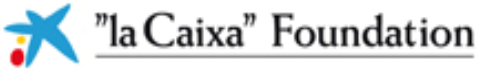

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Poster Session 1
Tuesday May 28 2013
6:35 PM – 8:00 PM

1. Letusa Albrecht
2. Alicia Arnott
3. Alyson M. Auliff
4. Celine Barnadas
5. Katherine E. Battle
6. Maria Bernabeu
7. Céline Borlon
8. Erika Braga
9. Cristiana Brito
10. Chetan Chitnis
11. Patchanee Chootong
12. Monika Chugh
13. Raul Chuquiyauri
14. Christopher Delgado
15. Christopher Delgado
16. Punith B. Devaraju
17. Manoj Duraisingh
18. Ingrid Felger
19. Miriam T. George
20. Eun-Taek Han
21. Jessica Hostetler
22. Rosalind E. Howes
23. Maria Kahn
24. Stefan H.I. Kappe
25. Kevin C. Kobylinski
26. Cristian Koepfli
27. Cristian Koepfli
28. Jessica T. Lin
29. Sandra March
30. Jutta Marfurt
31. James McCarthy
32. Michela Menegon
33. Florian Noulin
34. Francis B. Ntumngia
35. Tasanee Panichakul
36. Ruth O. Payne
37. Lalitha V. Ramanathapuram
38. Pilar Requena / Carlota Dobaño
39. Arturo Reyes-Sandoval
40. Amanda Ross
41. Manojit Roy
42. Juliana M. Sa
43. Lorenz von Seidlein
44. G. Dennis Shanks
45. Patrick L. Sutton
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47. Larry A. Walker
48. Anne-Marie Zeeman

** A list of presenters for Poster Session 2 is available on page 28 of this booklet **

Dissecting the Role of Rosetting in **Plasmodium vivax** *Malaria*

1. Letusa Albrecht, PhD[1], Stefanie C.P. Lopes, PhD[1], Andre M. Siqueira, MD[2,3], Carmen Bezerra-Fernandez, PhD[4], Hernando A. del Portillo, PhD[4], Marcos V.G. Lacerda, MD, PhD[2,3], Fabio T.M. Costa, PhD[1,2]; [1]Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP), Campinas, São Paulo, Brazil; [2]Fundação de Medicina Tropical Dr. Heitor Vieira Dourado, Manaus, Amazonas, Brazil, [3]Universidade do Estado do Amazonas, Manaus, Amazonas, Brazil, [4]Barcelona Centre for International Health Research, Hospital Clinic, University of Barcelona, Barcelona, Spain

Plasmodium vivax is the most prevalent parasite that causes malaria outside sub-Saharan Africa. For many years, malaria caused by *Plasmodium vivax* was believed to be a benign form of this disease; however recently findings of clinical complications are challenging this currently view. The pathogeneses of this parasite is still not fully understood. Nevertheless, as observed in *Plasmodium falciparum*, *Plasmodium vivax* can also cytoadhere to endothelial cell receptors and form rosettes. Rosette is a cytoadhesion phenotype in which an infected red blood cell can adhere to non-infected red blood cells. Although rosetting has been described for *Plasmodium vivax* more than 20 years ago, little is known about its aetiology and its role on *vivax* malaria infection. Thus, after analysing 38 *Plasmodium vivax* isolates from Brazil Amazon and we observed that the frequency of rosettes are significantly higher in mature parasites. All the isolates had enhanced the number of rosettes at the presence of autologous plasma compared to non-immune plasma. No association with anaemia or thrombocytopenia and rosetting formation was observed. Moreover, plasma heat-denaturation and filtration n 0.22 um did not interfere in rosetting formation, suggesting that neither complement nor platelets play a role. Interestingly, antibodies towards to VIR proteins were able to disrupt rosettes on *Plasmodium vivax* isolates suggesting that these surface antigens are involved on the rosetting formation. Taken together the results added more information about the biology of *Plasmodium vivax*.

2. ***Global Population Structure of the*** **Plasmodium vivax** ***Vaccine Candidates Apical Membrance Antigen 1 (AMA-1) and Merozoite Surface Protein 1 (MSP-1)***

Alicia Arnott[1], Ivo Mueller[2,3], Peter Siba[4], John C. Reeder[1,5], Alyssa E. Barry[3,6]; [1]Centre for Immunology, Burnet Institute, Melbourne, Australia, [2]Barcelona Centre for International Health Research, Barcelona, Spain, [3]Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, [4]Papua New Guinea Institute for Medical Research, Goroka, Papua New Guinea, [5]Monash University, Melbourne, Australia, [6]University of Melbourne, Parkville, Australia

Despite 40% of the world's population living at risk of *Plasmodium vivax* infection only two vaccine candidates targeting non blood-stage antigens have reached phase I clinical trials. The blood-stage antigens apical membrane antigen 1 (AMA-1) and merozoite surface protein 1 (MSP-1) represent promising vaccine candidates. These molecules are known to be highly polymorphic, however it remains unclear as to whether the extent and distribution of worldwide *Pvama1* and *Pvmsp1* genetic diversity might preclude vaccine coverage. The aim of this study was to investigate the population genetic structure of *Pvama1* and *Pvmsp1* in Papua New Guinea (PNG), and to compare the sequence data from PNG to published data from parasite populations of other *P. vivax* endemic areas worldwide. The complete *Pvama1* ectodomain and *Pvmsp1* C-terminal were amplified from 102 single-clone infections from PNG, and published *Pvama1* and *Pvmsp1* sequences were obtained from GenBank. Nucleotide and haplotype diversity was determined; signatures of balancing selection and non-synonymous single nucleotide polymorphisms (SNPs) with a minor allele frequency >10% were identified. Clusters of related haplotypes were identified using STRUCTURE and network analyses, and the geographical distribution of haplotypes investigated. Despite high levels of genetic diversity, these analyses were able to provide a valuable framework for the selection of a limited number of representative haplotypes that may cover a large proportion of this diversity. Immunological studies are needed to determine the breadth and magnitude of humoral immune responses induced by the selected haplotypes, and whether these responses are associated with clinical immunity to *P. vivax* infection.

3. *Functional Analysis of* Plasmodium vivax *Dihydrofolate Reductase-Thymidylate Synthase Genes through Stable Transformation of* Plasmodium falciparum

Alyson M. Auliff, PhD[1,2], Bharath Balu, PhD[3,4], Nanhua Chen, PhD[1], Michael T. O'Neil, PhD[5], Qin Cheng, PhD[1,2], John H. Adams, PhD[3]; [1]Australian Army Malaria Institute, Enoggera, Queensland, Australia, [2]University of Queensland, Brisbane, Australia, [3]Global Health Infectious Disease Research Program, University of South Florida, Tampa, Florida, United States, [4]SRI International, Virginia, United States, [5]Walter Reed Army Institute of Research, Silver Spring, Maryland, United States

Mechanisms of drug resistance in *Plasmodium vivax* have been difficult to study partially because of difficulties in culturing the parasite *in vitro*. This hampers monitoring drug resistance and research to develop or evaluate new drugs. There is an urgent need for a novel method to study mechanisms of *P. vivax* drug resistance. In this paper we report the development and application of the first *Plasmodium falciparum* expression system to stably express *P. vivax dihydrofolate reductase-thymidylate synthase (dhfr-ts)* alleles. We used the *piggyBac* transposition system for the rapid integration of wild type, single mutant (117N) and quadruple mutant (57L/58R/61M/117T) *pvdhfr-ts* alleles into the *P. falciparum* genome. The majority (81%) of the integrations occurred in non-coding regions of the genome; however, the levels of *pvdhfr* transcription driven by the *P. falciparum dhfr* promoter were not different between integrants of non-coding and coding regions. The integrated quadruple *pvdhfr* mutant allele was much less susceptible to antifolates than the wild type and single mutant *pvdhfr* alleles. The resistance phenotype was stable without drug pressure, while the level of resistance in parasites episomally expressing the *pvdhfr* quadruple mutant allele reduced with the decreasing copy number of episomes. All the integrated clones were susceptible to the novel antifolate JPC-2067. Therefore, the *piggyBac* expression system provides an important tool to investigate drug resistance mechanisms and gene functions in *P. vivax*.

4. *Retrospective Investigation of the Selection of Drug-Resistant* Plasmodium vivax *Parasites in Papua New Guinea*

Celine Barnadas, PhD[1,2,3], Elisheba Malau, BSc[2,3], Raksmei Keo[2], Lincoln Timinao, MSc[1], Sarah Javati, MSc[1], Peter M Siba, PhD[1], Timothy Davis, DPhil MBBS[4], Ivo Mueller, PhD[2,3,5]; [1]Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea, [2]Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, [3]University of Melbourne, Parkville, Victoria, Australia, [4]University of Western Australia, Perth, Australia, [5]Barcelona Centre for International Health Research, Barcelona, Spain

Plasmodium vivax resistance to chloroquine (CQ) was first described in 1989 in Papua New Guinea (PNG). In 2000, sulfadoxine-pyrimethamine was added to CQ and amodiaquine (AQ) as the treatment of first choice for *P. vivax* malaria. In 2005, the treatment failure rate ranged from 0% in East Sepik to 29% in Madang Province, indicating that drug-resistant parasites had been selected and that levels of resistance were not homogeneous across the country. We therefore aimed to characterize the selection and spread of resistant *P. vivax* parasites in these two PNG populations. Genotyping of *P. vivax* dihydrofolate reductase gene (*pvdhfr*) and multidrug resistance 1 (*pvmdr1*) potentially associated with resistance to pyrimethamine and 4-aminoquinolines, respectively, was performed on archived samples collected between 1991 and 2010 in East Sepik and Madang Provinces. Polymorphism of microsatellites in the flanking regions of these genes was characterized. Prevalence of wild-type *pvdhfr* isolates decreased from 95.6% in 1991–1992 to 52.4% in 2006 ($P<0.001$) while the quadruple mutant increased from 2.0% to 16.7% ($P=0.031$). In 2005, the prevalence of the quadruple mutant *pvdhfr* genotypes was significantly higher in Madang than East Sepik (52.2% vs 16.7%, $P=0.001$). Similarly, the *pvmdr1* 976F mutation putatively associated with CQ resistance was significantly higher in Madang (71.6% vs 26.2%, $P=0.001$). Preliminary sequence analysis of *pvdhfr* indicates at least three distinct origins of the quadruple mutant *pvdhfr* isolates in PNG.

5. *Global Geographical Variation in* Plasmodium vivax *Relapse Rate*

Katherine E. Battle, MSc[1], Samir Bhatt, DPhil[1], Peter W. Gething, PhD[1], J. Kevin Baird, PhD[2], Simon I. Hay, DPhil[1,3]; [1]Spatial Ecology and Epidemiology Group, University of Oxford, Oxford, United Kingdom, [2]Eijkman Oxford Clinical Research Unit, Menteng, Jakarta, Indonesia, [3]Fogarty International Center, National Institutes of Health, Bethesda, Maryland, United States

Plasmodium vivax has the widest geographic distribution of the human malaria parasites. Nearly 2.5 billion people live at risk of infection, which may cause severe disease or death. Control of *P. vivax* is complicated by its ability to relapse, weeks to months after initial infection. Risk and frequency of relapse varies among strains of *P. vivax*. Strains of tropical origin have multiple frequent (three to six weeks) relapses, while temperate strains have few or a single relapse six to 12 months following initial infection. The theory that relapse periodicity varies geographically and is timed to optimize opportunities for parasite transmission was assessed through a systematic review of reports of *P. vivax* relapse in patients not treated with primaquine. The association between relapse frequency and duration of transmission suitability in various regions was analyzed using meteorological variables and biological models. A map of relapse events plotted over a transmission suitability grid showed high relapse frequency predominantly in tropical regions with year round suitability and prolonged relapse in temperate regions with four to six month transmission "seasons". Statistical analyses supported an association between relapse frequency and duration of transmission. These geographic patterns do not clarify causation, but are consistent with the hypothesis that relapse frequency is an adaptation to optimize vector availability during periods of transmission. Relapse frequency may result from evolved responses to transmission season duration or arise from cues, such as triggers from other infections, correlated with *P. vivax* transmission periods. Regardless, these patterns are important to those working to treat, estimate and control the disease.

6. *Plasmodium vivax Subtelomeric Variant Proteins and Cytoadherence to the Human Spleen*

Maria Bernabeu, MSc[1], Mireia Ferrer, PhD[1,2], Stefanie C.P. Lopes, PhD[3], Richard Thomson, MD[1], Lorena Martin-Jaular, PhD[1], Aleix Elizalde, MD[1], Marcus V.G. Lacerda, MD, PhD[4], Fabio T.M. Costa, PhD[3], Carmen Fernandez-Becerra, PhD[1], Hernando A. del Portillo, PhD[1,5] ; [1]Barcelona Centre for International Health Research, Barcelona, Spain, [2]Centre d'études d'agents Pathogènes et Biotechnologies pour la Santé, Montpellier, France, [3]Instituto de Biologia, Universidade Estadual de Campinas, Campinas, Brazil, [4]Fundação de Medicina Tropical Dr. Heitor Vieira Dourado, Manaus, Amazonas, Brazil, [5]Institució Catalana de Recerca i Estudis Avançats, Barcelona, Spain

The lack of a continuous *in vitro* culture system for blood stages of *Plasmodium vivax* has severely limited our understanding of the molecular basis of pathology in this species. Noticeably, recent data have challenged the dogma that *P. vivax*-infected reticulocytes do not cytoadhere in the deep vasculature of internal organs. Here, we show that a transgenic line of *P. falciparum* expressing a VIR protein but not another transgenic line expressing a PvFAM-D protein, mediated adherence to human spleen fibroblasts. Spleen-adherence specificity was shown as neither transgenic line bound to human lung fibroblasts. To extrapolate these results to natural infections, adhesion experiments using *P. vivax*-infected reticulocytes obtained from human patients were performed on human spleen fibroblasts as well as to spleen cryosections. Results demonstrated adherence, albeit variable, among different isolates. Moreover, this adherence was inhibited by anti-VIR antibodies. These data reinforce the fact that cytoadherence is not exclusive of *P. falciparum* and challenges the dogma that the spleen is the evolutionary driven force for cytoadhesion of infected red blood cells in other organs for avoidance of spleen-clearance.

7. *Cryopreserved* Plasmodium vivax *and Reticulocytes from Cord and Adult Blood: Invasion and Short Term Culture*

Céline Borlon, PhD[1], Bruce Russell, PhD[2], Kanlaya Sriprawat, MSc[3], Annette Erhart, MD, PhD[1], Laurent Renia, PhD[4], François Nosten, MD-PhD[3,5], Anna Rosanas-Urgell, PhD[1], Umberto D'Alessandro, MD, PhD[1,6]; Institute of Tropical Medicine Antwerp, Belgium, [2]Yong Loo Lin School of Medicine, National University of Singapore, Singapore, [3]Shoklo Malaria Research Unit, Mae Sot, Thailand, [4]Singapore Immunology Network, Singapore; [5]Center for Clinical Vaccinology and Tropical Medicine, Oxford, United Kingdom; [6]Medical Research Council Unit, Fajara, The Gambia

The development of a *Plasmodium vivax in vitro* culture system is critical for the development of new vaccine, drugs and diagnostic tests. Though short term cultures have been successfully set up, their reproducibility in laboratories without direct access to *P. vivax* patients has been limited by the need of fresh parasite isolates. We have explored the possibility of using both frozen parasite isolates and frozen reticulocytes concentrated from cord blood to perform invasions. Additionally, we compared the ability of different sources of reticulocytes to sustain a short term culture of *P. vivax*. More than 50 invasion tests were performed according to the protocol previously developed. Short term culture of *P. vivax* in concentrated reticulocytes from cord, adult and hemochromatosis blood were started after a new invasion and supplemented with reticulocytes every 48h following the parasites life cycle. Invasion could be performed with similar efficiency for any of the combination (fresh/frozen reticulocytes and *P. vivax* isolates) used. Short term cultures of *P. vivax* couldn't be maintained over five days independently of the reticulocytes source. The invasion method can be easily replicated in laboratories outside endemic areas and can substantially contribute to the development of a continuous *P. vivax* culture. There is no improvements given by the source of reticulocytes used to initiate a short term culture.

8. *Epitope Mapping of* Plasmodium vivax *Merozoite Surface Protein 1 (PvMSP1): Identification of Novel Biomarkers to* P. vivax *Anemia*

Ingrid Oliveira, BS[1], Luiza Mourão, MSc[1], Adriana Fernandes, PhD[1], Ricardo Avila, PhD[1], Carlos Olórtegui, PhD[1], **Erika Braga**, PhD[1]; [1]Universidade Federal de Minas Gerais, Minas Gerais, Brazil

Identification of antigenic determinants in PvMSP-1, a potential candidate for an antimalarial vaccine, is necessary to reveal the precise nature of the peptides acting as main targets to naturally acquired antibodies and their possible relation to clinical features. The present study aims to identify in the PvMSP1 Sal1 strain possible biomarkers for protection and/or morbidity by Spot-Synthesis. The reactivity of antibodies to specific epitopes was quantified for each spot using the Image J 1.45s software. Background interferences were minimized by subtracting the mean values obtained for non-reactive area of the membrane from each spot images. Sera from anemic *P. vivax* patients recognized 459 peptides whereas non-anemic recognized only 282. Sera from *Plasmodium falciparum* patients with low (<7g/dL) or normal (>12g/dL) levels of hemoglobin were used to select peptides that are exclusive recognized by antibodies induced by *P. vivax*. The number of spots recognized by sera from *P. falciparum* patients was approximately 300 peptides. Interestingly, sera from anemic patients by other etiologies recognized 283 (normocytic anemia) and 257 (microcytic anemia) spots. Similarly, sera from non-malaria controls recognized only 252 spots. It is important to note that sera from anemic P. *vivax* malaria patients presented the higher reactivity considered at least 2-fold higher compared to the reactivity detected for other groups. Four peptides showed a good and exclusive capability for recognition of antibodies from P. *vivax* anemic patients. This finding could have potential relevance not only for serodiagnosis but also as a starting point for the characterization of mechanisms enrolled in *vivax* malaria pathogenesis.

9. *Genotyping* Plasmodium vivax *from Multiple-Clone Infections*

Aracele Souza, BS[1], Flávia Araújo, BS[1], Taís Sousa, PhD[1], Cor Fontes, PhD[2], Luzia Carvalho, PhD[1], **Cristiana Brito**, PhD[1]; [1]Centro de Pesquisas René Rachou, Fundação Oswaldo Cruz, Belo Horizonte, Minas Gerais, Brazil, [2]University Hospital Júlio Müller, Universidade Federal de Mato Grosso, Cuiabá, Mato Grosso, Brazil

Multiple *Plasmodium vivax* clones can often co-infect the same human host in endemic settings. The distinction of genetically different clones of parasites is essential because the competition among parasite clones into the host might drive parasite virulence and fitness. The aim of this study is to identify molecular markers able to genotype multiple-clone infections. For this purpose two approaches were used to do artificial multiple-clone admixtures: (i) Uncloned variants- PCR products from two variants of each marker were used (patients with similar parasitemia and leucocytes count); (ii) Cloned variants- PCR products were cloned into vectors (pGemT and PCR2.1). Artificial admixtures were performed using different amounts of DNA from each variant. After that, detection of multiple-clone infection was validated in field populations. For genotyping, we used as molecular markers microsatellites described by our group, tanden repeats, blocks 2 and 10 of MSP-1 and MSP3α. Our results showed that artificial mixtures using directly PCR products (approach 1) was better to distinguish rare variants than cloned-variants. Moreover, admixtures with different variant proportions were better detected using MSP-1 blocks 2 and 10 than the microsatellites. Using artificial admixtures we also analyzed different cut offs which allowed identification of the low frequent variant in multiple-clone infections. Altogether, these results suggested that encoding genes of polymorphic proteins are good markers to distighish multiple-clone infections.

10. *Process and Pre-Clinical Development of a Recombinant Vaccine for* Plasmodium vivax *Malaria Based on Duffy Binding Protein*

Rukmini Bharadwaj, PhD[1], Rushdi Shakri, PhD[1], Gaurav Pandey, PhD[2], Steve Reed, PhD[3], Darrick Carter, PhD[3], **Chetan Chitnis,** PhD[1]; [1]International Centre for Genetic Engineering and Biotechnology, New Delhi, India, [2]Malaria Vaccine Development Program, New Delhi, India, [3]Infectious Diseases Research Institute, Seattle, United States

Plasmodium vivax is completely dependent on interaction with the Duffy antigen receptor for chemokines (DARC) for invasion of human erythrocytes. The *P. vivax* Duffy binding protein (PvDBP) mediates interaction with DARC. The DARC receptor-binding domain lies in a conserved N-terminal cysteine-rich region of PvDBP referred to as region II (PvDBPII). Naturally acquired binding inhibitory antibodies against PvDBPII are associated with reduced risk of *P. vivax* infection. Moreover, antibodies against PvDBPII block erythrocyte invasion by *P. vivax. T*hese observations support the development of a blood stage vaccine against *P. vivax* based on PvDBPII. We have developed methods to produce recombinant PvDBPII in its correctly folded conformation. Recombinant PvDBPII was expressed in *E. coli* using a synthetic gene optimized for expression. Recombinant PvDBPII was collected from inclusion bodies, solubilized in 6M Guanidine HCl, refolded by rapid dilution and purified by ion exchange chromatography. Purified recombinant PvDBPII was characterized for identity, purity and functional activity using standardized release assays. Recombinant PvDBPII formulated with alhydrogel, alhydrogel + GLA-AF, GLA-SE, GLA-AF and R848-SE was used for immunization of Balb/C mice. In addition to priming immunization, booster immunizations were administered at days 0, 21 and 42 respectively. Sera collected at day 56 were tested for recognition of PvDBPII and inhibition of PvDBPII-DARC binding. GLA-SE and alhydrogel formulations of PvDBPII yielded the highest ELISA and binding inhibition tites. Sera raised against PvDBPII inhibited diverse polymorphic PvDBPII domains with similar efficiency suggesting that the binding site within PvDBPII is conserved. These data support the development of a recombinant vaccine for *P. vivax* based on PvDBPII, its receptor-binding dpomain.

11. *The Association of Duffy Binding Protein II Polymorphisms in* Plasmodium vivax *Isolates from Thailand*

Patchanee Chootong[1], Amy M. McHenry[2], Francis B. Ntumngia[3], Jetsumon Sattabongkot[4], John H. Adams[3]; [1]Faculty of Medical Technology, Mahidol University, Bangkok, Thailand, [2]Southwestern Adventist University, Texas, United States, [3]Global Health Infectious Disease Research Program, University of South Florida, Tampa, Florida, United States, [4]United States Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Plasmodium vivax Duffy Binding Protein II (DBPII) plays an important role in reticulocyte invasion and is a potential vaccine candidate against *vivax* malaria. However, polymorphism of DBPII is a potential challenge for the successful design of broadly protective vaccine. In this study, the genetic diversity of DBPII among Thai isolates were analyzed from Thai *P. vivax* infected blood samples and polymorphism characters were defined by MEGA4 program. Sequence analysis identified 12 variant residues that are common among Thai DBPII haplotypes with the variant D384G, L424I, H437 and I503K having the highest frequency. The variant D384K occurs in combination with either E385K or K386N/Q. Moreover, the variant L424I occurs in conjunction with W437R in most Thai DBPII alleles and these variants frequently occur in combination with variant I503K. The polymorphic patterns of Thai *vivax* isolates are defined into nine haplotypes, Thai DBL-1, -2, -3, -4, -5, -6, -7, -8 and -9. Thai DBL-2,-5, -6 are the most common DBPII variants. To study the association of Thai DBPII polymorphisms in the expression of antigenic character, the functional inhibition of anti-DBPII monoclonal antibodies against a panel of Thai DBL-2,-5,-6 variant was characterized by *in vitro* erythrocyte binding inhibition assay. The functional inhibition of anti-DBPII against variant Thai DBL 2,-5,-6 was significantly different. Our results indicate that amino acid mutation in variant Thai DBP-2, -5 and -6 change antigenic characters suggesting that the variant epitopes act as a target of anti-DBPII inhibitory antibodies. Therefore, to design protective vaccine in Thai residents should contain the variant B-cell epitope represent in Thai DBPII haplotypes.

12. *A Protein Complex Directs Hemoglobin to Hemozoin Formation in* Plasmodium

Monika Chugh, MSc[1], Kenneth D. Stuart, PhD[2], Pawan Malhotra, PhD[1]; [1]International Centre for Genetic Engineering and Biotechnology, India, [2]Seattle Biomedical Research Institute, Seattle, Washington, United States

Malaria parasites use hemoglobin (Hb) as a major nutrient source in the intra-erythrocytic stage during which “heme” is converted to hemozoin (Hz). The formation of Hz is essential for parasite survival as free heme is toxic to parasite. The study reports the presence of a ~200 kDa multi-protein complex in the food vacuole of *Plasmodium falciparum* that is required for Hb degradation and Hz formation. This complex contains falcipain 2/2’, plasmepsin II, plasmepsin IV, Histo Aspartic Protease (HAP) and Heme Detoxification Protein (HDP). The association of these proteins is evident from co-immunoprecipitation followed by mass spectrometry, co-elution from a gel filtration column and co-sedimentation in a glycerol gradient. An *in vitro* assay was developed using two of the proteins present in the complex. The results show that falcipain 2 and HDP associate with each other to efficiently convert Hb to Hz. The *in vitro* assay was also used to elucidate the modes of action of antimalarials: chloroquine and artemisinin. The genes in the complex are conserved among *Plasmodium* species. The *P. vivax* orthologs (percent identity/conservation) of the *P. falciparum* proteins in the complex are the PVX_091405 vivapain 2 (cysteine protease 60%/50%), PVX_086040 plasmepsin IV (aspartyl protease 72 %/71%) and PVX_118155 (hypothetical protein 77%/73% identity to HDP). Thus, this multi-protein complex appears to be conserved among *Plasmodium* species and additional knowledge of its function in these species may be useful to target the metabolic weak point in the life cycle of malaria parasite and guide the development of new antimalarials.

13. *Socio-Demographics and the Development of Malaria Elimination Strategies in the Low Transmission Setting*

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This analysis presents a comprehensive description of malaria burden and risk factors in Peruvian Amazon villages where malaria transmission is hypoendemic. More than 9000 subjects were studied in contrasting village settings within the Department of Loreto, Peru, where most malaria occurs in the country. *Plasmodium vivax* is responsible for more than 75% of malaria cases; severe disease from any form of malaria is uncommon and death rare. The association between lifetime malaria episodes and individual and household covariates was studied using polychotomous logistic regression analysis, assessing effects on odds of some vs. no lifetime malaria episodes. Malaria morbidity during lifetime was strongly associated with age, logging, farming, travel history, and living with a logger or agriculturist. Select groups of adults, particularly loggers and agriculturists acquire multiple malaria infections in transmission settings outside of the main domicile, and may be mobile human reservoirs by which malaria parasites move within and between micro-regions within malaria endemic settings. For example, such individuals might well be reservoirs of transmission by introducing or reintroducing malaria into their home villages and their own households, depending on vector ecology and the local village setting. Therefore, socio-demographic studies can identify people with the epidemiological characteristic of transmission risk, and these individuals would be prime targets against which to deploy transmission blocking strategies along with insecticide treated bednets and chemoprophylaxis.

14. *Dynamics and Population Structure of* Plasmodium vivax *Parasites under the Radical Cure Regimen in the Peruvian Amazon*

Christopher Delgado, MSc[1,2], Veronica E Soto-Calle, MD[2], Peter Van den Eede, PhD[3], Dionicia Gamboa, PhD[2], Angel Rosas, MD[2], Emmanuel Nji Abatih, PhD[3], Alejandro Llanos-Cuentas MD PhD[2], Jean-Pierre Van Geertruyden MD PhD[1], Annette Erhart MD PhD[3], Umberto D'Alessandro MD PhD[3,4]; [1]Antwerp University, Antwerp, Belgium, [2]Institute of Tropical Medicine Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru, [3]Institute of Tropical Medicine Antwerp, Belgium, [4]Medical Research Council Unit, The Gambia

Despite the large burden of *Plasmodium vivax*, little is known about its transmission dynamics. The study of the parasite population structure and dynamics give insights on particular epidemiological features of malaria in endemic areas. In a two-year cohort study in San Carlos community, a rural community of the Peruvian Amazon, we explored the population structure and spatio-temporal dynamics of *P. vivax.* Between March and December 2008, 37 *P. vivax* patients were treated radically with chloroquine and primaquine and followed up monthly for two years with systematic blood sampling. All samples were screened by microscopy and species-specific PCR for malaria parasites and subsequently all *P. vivax* infections genotyped using 15 microsatellites. 76% of the study participants experienced one or more recurrent *P. vivax* infections. 55% of the recurrences were sub-patent and asymptomatic. The *P. vivax* population displayed limited overall genetic diversity (He=0.49), high frequency of monoclonal infections (84%), presence of linkage disequilibrium (LD) and low probability of outbreeding (Psex<0.0001). Up to 20 unique haplotypes unequally distributed in four haplogroups were found. Haplotype replacement was reflected by spatio-temporal clustering and changes on the degree of LD and the genetic diversity after the first year of follow up (p<0.001). The clonal parasite population and haplotype clustering may influence the rate of recurrent episodes and the development of clinical immunity by the population (asymptomatic episodes).

15. ***Dynamics of Anti PvCSP-Antibodies During the First 28-Days Follow Up After Radical Cure Treatment in the Peruvian Amazon***

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After *Plasmodium vivax* sporozoites infection, *P.vivax* circumsporozoite protein (PvCSP) antibodies are produced by the human host. Hereby we present a preliminary study exploring the dynamics of anti PvCSP antibodies production within a group of patients followed weekly for 28 days after receiving a radical cure treatment in the Peruvian Amazon. Serological profiles of 112 individuals were analyzed by assessing IgG/IgM antibodies against the non-repeat region of PvCSP (long peptide N-term) by ELISA. A mixture model method was used to determine the cut off for negative/positive presence of antibodies anti-CSP. IgM/IgG were found in 18% of the samples on day 0, in 36% on day 7, in 32% on day 14, in 22% on day 21 and in 14% on day 28. We observed two characteristic patterns of anti PvCSP antibodies: The first one presenting two peaks, one appearing on the day 7 decreasing on day 14 and then other peak appearing on day 21 (in some individuals the apparition of the peak could start one week later). The second pattern described only one peak in most of the cases on day 7 or 14 (eventually on day 0). The knowledge of the dynamics of anti CSP antibodies may contribute on the development of a reliable tool added to the current approaches to study the efficacy of antimalarial drugs.

16. ***Epidemiology of Malaria in South-Western Region of India***

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Malaria is prevalent in many regions of the world, particularly in Africa, Southeast Asia, and South America, and is endemic in more than 106 countries. Despite the major malaria control efforts of the past decades, parasites have been constantly evolving and are becoming drug resistant globally. India accounts for about one third of all malaria cases in Southeast Asia. In Southwestern region of India, malaria is highly prevalent in Mangalore city and surrounding towns. However, there have been no systematic studies to understand malaria epidemiology, drug resistance, and pathology. In this study, analysis of the available epidemiological data indicates that predominantly *Plasmodium vivax* and to a less extent *P. falciparum* malaria persists throughout the year with moderate levels of mixed infections in Mangalore. During 1991 to 1994, nearly almost all the reported malaria cases were caused by *P. vivax* alone with little or no *P. falciparum* infections. However, from 1995 onwards, there has been a gradual increase in the extent of *P. falciparum* infections and currently it is in the range of 10–30%. Analysis of month-wise infection rate shows that peak infections occur during the monsoon rainy season (July and August). The data also suggests that, in recent years, there has been an increase in *P. falciparum* to *P. vivax* infection ratios and in the number of *P. vivax* severe malaria cases in Mangalore. We are currently focusing to study the pathophysiology of malaria, clinical presentations, drug resistance, and pregnancy malaria with main emphasis on *P. vivax*.

17. *Adaption of* Plasmodium knowlesi *to the Growth in Older Human Red Blood Cells: Can* Plasmodium vivax *Do the Same?*

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A major impediment to the establishment of continuous *in vitro* culture for *Plasmodium vivax* has been the restriction of parasite invasion and growth to the youngest of human red blood cells known as reticulocytes. We have studied the macaque malaria parasite *Plasmodium knowlesi* that has recently emerged as an important zoonosis in Southeast Asia. Infections are typically mild but can cause severe disease, achieving parasite densities similar to fatal *Plasmodium falciparum* infections. We show that a primate-adapted *P. knowlesi* parasite proliferates poorly in human blood due to a strong preference for young red blood cells. We establish a continuous *in vitro* culture system by using human blood enriched for young cells. Mathematical modelling predicts that parasite adaptation for invasion of older red blood cells is a likely mechanism leading to high parasite densities in clinical infections. Consistent with this model, we find that *P. knowlesi* can adapt to invade a wider age range of red blood cells, resulting in proliferation in normal human blood. Indeed, such cellular niche expansion may increase pathogenesis in humans and will be a key feature to monitor as *P. knowlesi* emerges in human populations. We will discuss the implications of our results and approaches for the culture adaptation of *P. vivax*.

18. *Improving the Molecular Detection of* Plasmodium vivax*: DNA- versus RNA-Based Detection of Blood Stages in Combination with Gametocytes*

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Microscopic detection of *Plasmodium vivax* suffers from low parasite densities, generally 10-fold lower than *P. falciparum*. Molecular detection of *P. vivax* likewise is more affected by random presence or absence of any template in the tested DNA aliquot when analyzing very low parasitaemia samples. We exploited the fact that *P. vivax* carries only five copies of the 18SrRNA marker gene/parasite, whereas 18SrRNA transcripts range from thousands to millions copies/parasite. We compared DNA- versus RNA-based detection in 315 5-9 yr old Papua New Guinean children. qPCR and qRT-PCR were performed for species determination targeting 18SrRNA genes or transcripts and for gametocyte detection using markers pvs/pfs25. 19.6% of DNA samples were *P. vivax* positive. RNA-based positivity was twice as high (38.4%). The discrepancy between both measures was less pronounced for *P. falciparum* with a DNA-based prevalence of 14.1% versus 24.1% RNA-based. Prevalence by light microscopy was substantially lower (5.4% for *P. falciparum* and 12.8% for *P. vivax*). Because most parasite carriers were asymptomatic, infections likely harbored low parasite densities around the detection limit of microscopy. Carriage of *P. vivax* gametocytes is increasingly used to measure transmission intensity, because of the simplicity of detection in host blood by targeting gametocyte-specific transcripts. For studies requiring simultaneous molecular quantification of sexual and asexual parasite stages, 18SrRNA-based prevalence is feasible without extra efforts or costs. Yet, for optimal comparability and harmonization with global molecular prevalence data, and by acknowledging the robustness of DNA-based assays, we propose reporting DNA-based parasite prevalence together with detection of pfs/pvs25 transcripts.

19. *Defining Minimal Reactive Epitopes on the Surface of* Plasmodium vivax *Duffy Binding Protein Reactive with Neutralizing Monoclonal Antibodies*

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The *Plasmodium vivax* Duffy Binding protein (*Pv*DBP) is a vital ligand for blood-stage development making the molecule an attractive vaccine candidate for inclusion in a vaccine designed to protect against *P. vivax* malaria. Similar to other blood-stage vaccine candidates, DBP allelic variation eliciting a strain-specific immunity may be a major challenge for development of a broadly effective vaccine against *P. vivax* malaria. To understand the nature and location of epitopes that can be the target of neutralizing anti-DBP inhibition, we generated and characterized a panel of anti-DBP monoclonal antibodies (mAbs) and functionally analyzed their reactivity to a panel of allelic variants. Quantitative analysis by ELISA determined that some monoclonals reacted strongly with epitopes conserved on all DBP variants tested, while reactivity of other monoclonals was allele-specific. Crystal structure of *Pv*DBPII shows that it consists of two α-helical bundles with an antiparallel β-hairpin near the N-terminus and may be assigned into three sub-domains delineated by six disulphide bonds. Using a phage display approach to express these individual sub-domains and combinations of sub-domains in their correctly refolded and disulphide bonded conformation, we mapped the epitopes of the monoclonal antibodies to primarily subdomain III. Finer epitope mapping is being achieved using both a random peptide library and a gene fragment library of *Pv*DBPII displayed on phage. Ultimately information derived from these analyses will contribute to the design and assessment of this antigen for inclusion in an asexual blood-stage vaccine designed to protect against malaria caused by *P. vivax*.

20. PvMSP1P, Merozoite Surface Protein 1 Paralog, Is a Novel Erythrocyte-Binding Ligand of Plasmodium vivax

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Merozoite surface protein 1 of *Plasmodium vivax* (PvMSP1), a glycosylphosphatidylinositol-anchored protein (GPI-AP), is a malaria vaccine candidate for *P. vivax*. The paralog of PvMSP1, named *P. vivax* merozoite surface protein 1 paralog (PvMSP1P; PlasmoDB PVX_099975), gene was recently identified and predicted as a GPI-AP. The similarities in genetic structural characteristics between PvMSP1 and PvMSP1P (e.g., size of open reading frames, two epidermal growth factor-like domains, and GPI-anchor motif in the C-terminus) led us to study this protein. In the present study, different regions of the PvMSP1P protein, demarcated based on the processed forms of PvMSP1, were expressed successfully as recombinant proteins [i,e., 83- (A, B, and C), 30, 38, 42, 33, and 19 fragments]. We studied the naturally acquired immune response against each fragment of recombinant PvMSP1P and the potential binding ability of each fragment to erythrocytes. The N-terminal (83A) and two C-terminal fragments (33 and 19) reacted strongly with sera from *P. vivax*-infected patients, with 50–68% sensitivity and 95–96% specificity, respectively. Due to colocalization of PvMSP1P with PvMSP1, we supposed that PvMSP1P plays a similar role as PvMSP1 during erythrocyte invasion. An in vitro cytoadherence assay showed that PvMSP1P, especially the C-terminal 19-kDa region, could bind to erythrocytes. We also found that human sera from populations naturally exposed to vivax malaria and antisera obtained by immunization

using the recombinant molecule PvMSP1P-19 inhibited in vitro binding of human erythrocytes to PvMSP1P-19. These results provide further evidence that the PvMSP1P might be an essential parasite adhesion molecule in the *P. vivax* merozoite and is a potential vaccine candidate against *P. vivax*.

21. *High Throughput Characterization of* Plasmodium vivax *Invasion Antigen Library*

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A vaccine targeting the illness-inducing blood stage of parasite development is an essential component of any worldwide malaria eradication campaign, but major gaps in our understanding of *Plasmodium vivax* biology, including critical events during blood-stage infection, hinder the search for a universal vaccine. Currently, only a single receptor-ligand interaction is known, that between *P. vivax* Duffy Binding Protein (PvDBP) and its cognate erythrocyte receptor, DARC, and strain-specific immune responses make PvDBP a challenging vaccine target. We are carrying out a comprehensive study of *P. vivax* proteins that mediate reticulocyte binding and invasion in order to identify additional vaccine candidates. As a first step, we generated a library of full-length *P. vivax* proteins to test for erythrocyte binding and immunoreactivity. We mined existing *P. vivax* microarray data and homology comparisons with *P. falciparum* to produce a list of 36 candidate merozoite proteins, the majority of which are predicted to localize at the merozoite surface, micronemes or rhoptries. The selected candidates were codon optimised for optimal expression in the HEK293E cell system, which has been successfully used for expression of full-length *P. falciparum* invasion ligands such as PfRH5. 26 proteins expressed at usable levels. Known or predicted functions, such as the interaction between merozoite surface proteins Pv12 and Pv41, were confirmed. Pilot immunoreactivity screens using 21 pairs of acute and convalescent sera from Cambodian patients with *P. vivax* malaria showed that 6/6 antigens tested are variously recognized by IgG. Initial *P. vivax* protein expression data, protein interaction data and seroreactivity data will be presented.

22. *G6PD Deficiency Prevalence and Genetic Variants: Implications for* Plasmodium vivax *Primaquine Radical Cure*

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Glucose-6-phosphate dehydrogenase deficiency (G6PDd) prohibits widespread use of primaquine. This genetic enzyme deficiency leaves red blood cells susceptible to oxidative stress, and exposure to primaquine may trigger life-threatening haemolysis in G6PDd individuals. However, primaquine is essential to malaria elimination because it is the only drug active against the dormant liver-stages of *Plasmodium vivax*, which may otherwise relapse into multiple clinical episodes in the months following infection. Safe access to primaquine must be increased to reach elimination targets, and an evidence-base to assess risks from G6PDd is a key step towards this. Here, we examine the spatial epidemiology of G6PDd: its overall prevalence and the distribution of its genetic variants which have important implications for its clinical severity. We developed a Bayesian geostatistical framework to predict a continuous map of G6PDd prevalence. The allele frequency of G6PDd was estimated at 8.0% across populations in malarious countries, with highest rates in sub-Saharan Africa. G6PDd genetic variants showed stark geographic patterns, with greatest heterogeneity and admixture among Asian populations east of India, where multiple variants are commonly polymorphic. Together, these datasets provide a qualitative indication of the haemolytic risks associated with widespread use of primaquine for *P. vivax* radical cure. However, a robust evidence base for understanding haemolysis is essential to allow quantitative predictions of risk. Potential sources of such evidence are discussed. Given the absence of a practical test for G6PDd-associated haemolytic risk in point-of-care settings, epidemiological evidence of these risks is necessary towards improving safe access to primaquine.

23. *Preservation of Intra-Erythrocytic Glucose-6-Phosphate Dehydrogenase Activity through Cryopreservation*

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common human enzyme deficiency. It is characterized by abnormally low levels of G6PD activity. Individuals with diminished G6PD activity are susceptible to cellular oxidative damage, and anti-malarial drugs such as those in the 8-aminoquinolone group (*e.g.,* Primaquine, Pamaquine, and Tafenoquine) can cause acute hemolysis in people with G6PD deficiency. Because of this risk it is imperative to identify individuals with G6PD deficiency prior to administering these anti-malarial agents. As such, there is a need for the development and evaluation of point-of-care G6PD deficiency screening tests suitable for areas of the developing world where malarial treatments are frequently administered. The development and evaluation of new G6PD tests will be greatly assisted with the availability of specimen repositories. We evaluated cryopreservation of erythrocytes as a means to preserve G6PD activity. Blood specimens from 31 patients including 10 specimens with Normal G6PD activity, three with intermediate activity and 18 specimens with deficient activity were cryopreserved for up to six months. We show that G6PD activity in these specimens is preserved as determined by quantitative assays, and qualitative assays. Furthermore we show that the mosaic composition of red blood cells in heterozygous women is also preserved as demonstrated by flow cytometry. We present a methodology for establishing a specimen panel for evaluation of G6PD tests. The availability of G6PD tests is a critical bottleneck to broader access to drugs that confer radical cure of *Plasmodium vivax*, a requirement for elimination of malaria.

24. *A Human Liver-Chimeric Mouse Model Supports* Plasmodium vivax *Liver Stage Development*

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The lack of a small animal model has prevented progress in understanding unique aspects of *Plasmodium vivax* biology, particularly liver stage development, persistence and relapse. We report here that the FRG mouse engrafted with human hepatocytes (huHep) is highly susceptible to *P. vivax* sporozoite infection and supports the development of *P. vivax* liver stages. This model in conjunction with a set of novel organelle-specific polyclonal and monoclonal antibodies allowed a detailed analysis of *P. vivax* liver stage development *in vivo*. Sporozoite infections performed with different Thai field isolates demonstrated robust schizont growth and development over a one-week period. A highly distinct population of small, non-replicating forms (hypnozoites?) that persisted over time was observed in *P. vivax*-infected livers but never in *P. falciparum*-infected livers. These forms exhibited a single nucleus, mitochondrion and apicoplast, and were ensconced in a parasitophorous vacuole. The effect of primaquine on the cellular integrity of liver stages will be presented. The robust infection of FRG huHep livers with replicating and non-replicating forms enables the testing of biologically informed hypotheses concerning the nature and mechanisms of hypnozoite formation and persistence, which have remained elusive. *In vivo* infections utilizing the FRG huHep model will also accelerate the discovery of novel drugs for the radical cure of *P. vivax*.

25. *The potential of Ivermectin Mass Drug Administration to Humans for* Plasmodium vivax *Control in Southeast Asia: Preliminary Evidence*

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Ivermectin, in the laboratory, has been demonstrated to: reduce *Anopheles gambiae* s.s. survivorship and fecundity, delay re-feeding, cause a knockdown effect and delay recovery, multiple ivermectin-containing blood meals compounds mortality, and inhibits the development of *Plasmodium falciparum* in *An. gambiae* when ingested before, concurrently with, or after parasites. Furthermore, ivermectin mass drug administration to humans in Senegal was shown to reduce *An. gambiae* survivorship and reduce the proportion of *P. falciparum*-infectious *An. gambiae*. *Plasmodium vivax* elimination from Southeast Asia will require novel human and vector targeted control measures. Preliminary evidence indicates that ivermectin is lethal for *Anopheles dirus* s.s. (LC_{50} = 29.38 ng/ml) and *Anopheles minimus* (LC_{50} = 5.18 ng/ml) at human relevant concentrations. A single trial indicated that ivermectin co-ingested with *P. vivax* by *An. dirus* significantly reduced the number of developing oocysts across a range of human relevant ivermectin concentrations. Future research plans include determining: LC_{50} values for other *P. vivax* vectors including *Anopheles sawadwongporni* and *Anopheles maculatus*, if ivermectin delays vector re-feeding in Southeast Asian malaria vectors, determine the extent that ivermectin inhibits *P. vivax* sporogony in *An. dirus* and *An. minimus*, and determine the duration that ivermectin treated humans reduce the survivorship and delay re-feeding of *An. dirus*. Ultimately, these findings will be used to justify whether or not ivermectin mass drug administration to humans in Southeast Asia could provide suppression of *P. vivax* transmission.

26. *Molecular Epidemiology of* Plasmodium vivax *in the World's Highest Transmission Setting*

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On the Papua New Guinean north coast *Plasmodium vivax* reaches the world's highest prevalence: in several studies 30–65% of children harbored *P. vivax*. To describe molecular epidemiological parameters in this setting, 264 children aged 1–4.5 were followed over 16 months. Every two months they were bled twice within 24 hours. Additional samples derived from passive case detection. *P. vivax* clones were genotyped from all samples using highly polymorphic markers. Overall *P. vivax* prevalence was 53%. 73% of *P. vivax* carriers harbored multiple-clone infections with a mean multiplicity of infection (MOI) of 2.8. Analysis of samples collected 24 hours apart showed that only 6–9% of parasite-positive children were missed when considering a single day. However, 17–31% of all clones (depending on the molecular marker) were missed on a single day; combining paired samples increased MOI to 3.4 clones/individual. Children acquired 16 new blood-stage clones per year-at-risk, compared to six *P. falciparum* clones. Bednet use lead to a 50% reduction in acquired *P. vivax* clones. This, however, did not influence incidence. Force of blood-stage infection did not change with age, thus the three-fold drop in clinical *P. vivax* incidence observed over the age range in the cohort was the result of fast acquisition of immunity. Population genetic characteristics of parasites from this cohort were compared to *P. vivax* populations across Papua New Guinea and Solomon Islands. In sharp contrast to regions of lower endemicity, absence of pronounced population genetic structure and no sign of inbreeding were observed.

27. *Prevalence of* Plasmodium vivax *and* Plasmodium falciparum Gametocytes in a High Transmission Setting

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Understanding human to mosquito transmission is crucial to control malaria. *Plasmodium vivax* gametocytes are short-lived and appear shortly after infection and before the onset of clinical disease while *P. falciparum* gametocytes develop within seven to 15 days after first appearance of blood-stage parasites and persist longer. It is unclear how overall prevalence, age and disease status of human hosts influence transmission. In a cross-sectional survey in 2010 in an area of intense, yet declining malaria transmission in Papua New Guinea, 2121 blood samples were collected from participants of all ages. *Plasmodium* species were detected by qPCR assays and gametocytes by reverse-transcriptase PCR of markers *pvs25* and *pfs25*. Prevalence by light microscopy and PCR was 4.8% and 13% for *P. vivax* and 6.2% and 17% for *P. falciparum*. *P. malariae* and *P. ovale* were rare with 28 and two positive samples. Gametocytes were detected in 49% of *P. vivax* and 64% of *P. falciparum* positive samples. Parasite density was considerably higher for Pf (geometric mean 51 parasites/ul) than Pv (1 parasite/ul) and in gametocyte rt-PCT positive vs. negative individuals for both species (Pf: 81 vs. 22 parasites/ul, Pv: 2.3 vs. 0.3 parasites/ul). In gametocyte positive individuals total parasite numbers and gametocyte counts correlated moderately for *P. vivax* (Spearman's r_s=0.50) but not for Pf (r_s=0.16). The higher proportion of samples positive for *P. falciparum* than *P. vivax* gametocytes was unexpected. It could reflect either a real biological difference and/or limited sensitivity of our current molecular methods to detect gametocytes in very low density samples.

28. *Using Ion Torrent Amplicon Deep Sequencing to Assess In-Host* Plasmodium vivax *Genetic Diversity in Cambodia*

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Plasmodium vivax infections are often polyclonal, with infected persons in endemic areas harboring more than one genetic variant. Next generation sequencing has advantages over standard genotyping methods for characterizing this in-host genetic diversity, including the ability to detect minority variants, determine variant frequency, and construct haplotypes. Using the Ion Torrent sequencing platform, we deep sequenced 133bp of the polymorphic *P. vivax* merozoite surface protein-1 gene in 98 *vivax* isolates collected from a cohort of 65 Cambodians, obtaining on average 3,460x coverage. All samples were amplified and sequenced in duplicate, with only haplotypes present in both samples at ≥0.5% frequency and not determined to be chimeras counted as unique variants. In total, we identified 50 unique *pvmsp1* haplotypes within 65 individuals. If only the majority variant in each isolate was detected, 34/50 (78%) haplotypes would have been missed since they only existed as minority variants. Most (83/98) isolates were polyclonal, containing on average 3.7 variants (range 1–13), with isolates from recurrent infections similarly complex. Among the 22 persons with recurrences, only six (27%) displayed genotypes at recurrence that contained the same dominant variant seen previously. However, taking into account minority variants, 14 (64%) persons displayed homologous recurrences. Our results highlight an incredible genetic diversity existing below the surface of clinical *vivax* infections in Cambodia. This diversity can be exploited to better characterize relapses in endemic settings. We hypothesize that simultaneous hypnozoite activation and relapse of multiple clones promotes genetic diversity, making relapse an important mechanism for maintaining *P. vivax* genetic diversity.

29. *A Microscale Human Liver Platform that Supports the Liver Stages of* Plasmodium falciparum *and* Plasmodium vivax

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The *Plasmodium* liver stage is an attractive target for the development of anti-malarial drugs and vaccines, as it provides an opportunity to interrupt the life cycle of the parasite at a critical early stage before disease pathology or advanced infection have occurred. However, targeting the liver stage has been difficult. Undoubtedly, a major barrier has been the lack of robust, reliable and reproducible *in vitro* liver stage cultures. Here, we establish the liver stages for both *Plasmodium falciparum* (*P. falciparum*) and *P. vivax* in a microscale human liver platform of primary hepatocytes. Our platform is based on the interaction of fresh or cryopreserved *P. falciparum* or *P. vivax* sporozoites with cryopreserved human hepatocytes organized amongst supportive stromal cells in micropatterned cocultures (MPCC), which we have previously shown to stabilize the hepatocyte phenotype for 4-6 weeks. Using this system, we have successfully recapitulated the full liver stage of *P. falciparum* including the release of infected merozoites and subsequent infection of overlaid human erythrocytes and the establishment of small forms in late liver stages of *P. vivax*. Finally, we validate the potential of this automation-capable platform as a tool for medium-throughput anti-malarial drug screening and vaccine development.

30. *Potent ex vivo Activity of Pyronaridine, Naphthoquine, and Methylene Blue Against Chloroquine Resistant Field Isolates of* Plasmodium falciparum *and* Plasmodium vivax

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In vitro drug susceptibility testing of *Plasmodium falciparum* is used routinely to screen for antimalarial drug activity free from confounding effects of host immunity and drug pharmacokinetics. Despite the lack of a continuous *ex vivo* culture system for *P. vivax*, the revised, field-based schizont maturation test has proven utility in measuring the comparative *ex vivo* schizonticidal activity of novel and standard antimalarials against field isolates to help prioritise the development of new therapies which retain efficacy to both species of malaria. The 4-aminoquinolines naphthoquine (NQ) and pyronaridine (PYR) have been introduced recently as a partner drugs for anti-malarial combination therapy (ACT). Methylene blue (MB) is an old antimalarial, but was abandoned because of its side effects. We assessed the anti-malarial *ex vivo* activity of NQ, PYR and MB against multidrug-resistant *P. vivax* field isolates and showed good *ex vivo* efficacy (median IC_{50}, [range]) for NQ (3.6 nM [1.5–34.2]), PYR (2.4 nM [0.7–11.4]), and MB (1.2 nM [0.7–1.6]). Correlation patterns between NQ, PYR and other quinoline-based compounds chloroquine, amodiaquine, piperaquine, and mefloquine were different between the two malaria species. Studies investigating the stage-specific activity of these compounds are still ongoing. The potent *ex vivo* activity of NQ, PYR and MB against both *P. falciparum* and *P. vivax* highlights a promising role as partner drugs in ACTs in geographical locations where both species are endemic. In addition, these *ex vivo* data provide essential phenotypic information for complementary molecular studies elucidating the genetic basis of resistance in *P. vivax*.

31. *Experimentally Induced Blood-Stage* Plasmodium vivax *Infection in Healthy Volunteers*

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Major impediments to develop vaccines and drugs for *Plasmodium vivax* malaria are the inability to sustain this species in culture, and the extreme difficulty in undertaking clinical research by experimental infection. Here we report the collection and detailed characterization of a *P. vivax* parasite bank, from a 49 year old woman who presented to a hospital in Brisbane, Australia with *P. vivax* infection, and the subsequent conduct of an experimental infection study in two volunteers using this isolate. The donor made a full recovery from malaria following bank collection, which tested negative for all agents routinely screened for in blood donations. DNA sequence analysis of the isolate suggested that it was clonal, but highlighted the presence of molecular markers indicative of pyrimethamine resistance, and significant divergence from strains so far sequenced. Two subjects inoculated with the isolate developed parasitemia, as detected by PCR, on days eight and nine, followed by onset of symptoms of malaria and detection of parasites on peripheral blood smear on day 14, when they were treated with artemether-lumefantrine, with rapid clinical and parasitological response. Transcripts of the parasite gene *pvs25* that is expressed in gametocytes were first detected on days 11 and 12. This experimental system results in *in vivo* parasite growth, likely infectious to mosquitos. It offers the opportunity to undertake studies previously impossible in *P. vivax* that will facilitate a better understanding of the pathology of *vivax* malaria and the development of much needed drugs and vaccines.

32. *Large Extent of Genetic Diversity in* Plasmodium vivax *Isolates Infecting Pregnant Women from Manaus, Amazonas State, Brazil*

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In 2008, a research project funded by the European Commission was launched to estimate the burden of *Plasmodium vivax* infection in pregnancy as well to determine the population structure of *P. vivax* isolates in five endemic countries: Brazil, Colombia, Guatemala, Papua New Guinea and India (grant 7FP-HEALTH-201588). The present study aimed to investigate the genetic diversity of *vivax* populations in pregnant women. To do so, we analyzed seven polymorphic microsatellites (MS) previously used in studies of the genetic diversity of *P. vivax* isolates from different countries. Blood samples were collected from pregnant women at different time points during pregnancy and from non-pregnant women (control samples) from Manaus, Amazonas State (Brazil). MS loci were amplified by PCR and amplicon lengths were determined on an automated DNA-sequencer. Genotyping analysis of 70 *P. vivax* isolates showed a number of alleles across loci ranging from seven (MS1 and MS3) to 29 (MS8), with an average of 15.7 alleles per isolate. The genetic diversity of the studied population, calculated by the virtual H_E of each locus, ranges from 0.70 (MS1) to 0.95 (MS8), with a mean H_E of 0.85. This value is higher to the one previously reported in 11 *P. vivax* isolates from the same area and analyzed by eleven distinct MS loci (mean H_E = 0.77). The average H_E value obtained in this study indicates that, in spite of a low incidence of *vivax*-associated pregnancy malaria, pregnant women carry-on parasite populations with a conspicuous level of genetic diversity.

33. *Influence of Hematopoietic Stem Cell Source to Produce Reticulocytes for the* In Vitro *Culture of* Plasmodium Falciparum

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One of the main bottlenecks for the establishment of a *Plasmodium vivax in vitro* culture is the preference of *P. vivax* for reticulocytes, representing only one percent of total red blood cells. To tackle this problem, a source of reticulocyte is necessary. One of the solutions is to produce reticulocyte derived from stem cells. It has been previously shown that reticulocytes obtained after derivation of cord blood hematopoietic stem cells (HSC) could be efficiently used for *P. vivax* invasion assays. Stem cell derived reticulocytes contain foetal haemoglobin which may affect parasite development as previously proposed. However, whether foetal haemoglobin impairs the growth of *Plasmodium* parasites remains to be solved and needs to be addressed to further develop *P. vivax* culture technics. The aim of our study is to investigate whether several sources of hematopoietic stem cells, such cord blood (CB), peripheral mononuclear cells (PBMC) and bone marrow (BM), can be used to produce reticulocytes for *P. vivax* culture. Using a 14 days protocol, adapted from Giarratana *et al*., 2005, we were able to produce approximately 18% reticulocytes from CB, 25% from BM and 26% from PBMC. In order to investigate the effect of foetal haemoglobin on the growth of *Plasmodium* parasites, we are currently performing invasion assays and short term culture with *P. falciparum* using stem cells derived reticulocytes. Preliminary results did not show any significant difference after seven days of *in-vitro culture.* Future plans include the use of stem cells derived reticulocytes for *P. vivax* invasion assays.

34. *Immunogenicity of a Synthetic Antigen Based on the Ligand Domain of the Plasmodium vivax Duffy Binding Protein*

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The *Plasmodium vivax* Duffy binding protein region II (DBPII) is an essential ligand for reticulocyte invasion, thereby giving this molecule priority as a prime target for vaccine development against blood-stage *vivax* malaria. DBPII is polymorphic, reflecting a mechanism consistent with selective immune pressure that tends to compromise vaccine efficacy associated with strain-specific immunity. We hypothesized that the polymorphic residues, which are not functionally important for erythrocyte binding but flank the receptor binding motif of DBPII, comprise variant epitopes that tend to divert the immune response away from more functionally conserved epitopes. In this study, we evaluated the immunogenicity of a synthetic DBPII allele termed DEKnull, which lacks an important immunodominant variant epitope and evaluated its potential to produce an immune response that is relevant to naturally occurring DBPII alleles. We demonstrated that the DEKnull antigen retained erythrocyte-binding activity and elicited functional antibodies against shared neutralizing epitopes on native DBPII alleles. Anti-DEKnull antibody titers were lower but produced more broadly neutralizing anti-DBPII inhibitory responses, than single native DBPII alleles. A DBPII vaccine targeting immune responses to more conserved epitopes that are targets of neutralizing immunity may avoid induction of strain-specific immunity and enhance functional inhibition against a broader range of DBPII variants. Optimization of the DEKnull antigen is necessary to enhance its immunogenicity and induce broadly neutralizing immunity.

35. Inhibition of Erythroid Development by Plasmodium vivax

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Anemia has frequently been associated with severe malaria and contributes to the morbidity and mortality effects of the disease. Reports of *Plasmodium vivax* malaria in bone marrow from severely anemia patients exhibited dyserythropoiesis. However, the mechanism involving in induction of anemia in vivax malaria is poorly understood. Here, hematopoietic stem cells /$CD34^+$ cells from normal human cord blood were subjected to study the inhibition of erythropoiesis by *P. vivax*. Intact or lysed *P. vivax*-infected erythrocytes (IEs) isolated from patient blood were added to cultures of $CD34^+$ cells/erythroid progenitor cells. Results showed both intact and lysed IEs inhibited erythroid growth 57 and 59 %, respectively, compared with controls containing without IEs. The reduction of erythroid growth was not significantly greater by intact IEs when compared with lysed IEs. Interestingly, *P. vivax* inhibited not only erythroid growth, but also erythroid development, as determined by reduction of cells expressing 28.1 % of glyphorin A and 43.9 % of CD 71. The susceptibility to the inhibitory effect of *P. vivax* was decreased when erythroid cells were mature. Moreover, vivax parasites pertured the division of erythroid cells, as measured by the Cytokinesis Block Proliferation Index, which was reduced to 1.35 (p-value < 0.01) from a value of 2.08 in controls. Neither TNF-α nor IFN-γ was detected in the culture medium of erythroid cells treated with IEs indicating that impaired erythropoiesis was independent of these cytokines. Cell death was also found at similar levels in cultures with or without IEs. This finding suggests *P. vivax* parasites directly inhibit erythropoiesis leading to ineffective erythropoiesis and highlights the potential of *P. vivax* to cause severe anemia.

36. *Clinical Development of a Blood-Stage* Plasmodium vivax *Vaccine*

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Plasmodium vivax is increasingly recognised as a significant cause of morbidity and economic loss worldwide, with some attributable mortality. Control of *P. vivax* is challenging, with re-emergence in areas where it has previously been eliminated. Little progress has been made previously in development of a *P. vivax* vaccine with only two *P. vivax* antigens reaching Phase I clinical trials. We report here on a Phase Ia clinical trial currently underway in Oxford using simian adenovirus ChAd63 and MVA encoding the *P. vivax* antigen PvDBP (Duffy-binding protein region II) in a heterologous prime-boost regimen. The viral vectors used in this study have been tested extensively in trials of *P. falciparum* vaccines, and demonstrate excellent safety and immunogenicity. Invasion of red blood cells by *P. vivax* requires interaction between the parasite ligand Duffy-binding protein and its host receptor, the Duffy antigen receptor for chemokines (DARC), on the erythrocyte surface. Unlike *P. falciparum* which can utilise multiple redundant pathways for human erythrocyte invasion, the interaction between PvDBP and DARC is vital for *P. vivax*, making PvDBP a very promising antigen for vaccine development. Pre-clinical studies have shown that the viral vectors ChAd63 and MVA expressing PvDBP induce strong antibody immunogenicity and functional activity to block binding of PvDBP to DARC *in vitro*. This Phase Ia study examines the safety and immunogenicity of ChAd63/MVA PvDBP heterologous prime-boost in twenty four healthy volunteers. If the vaccine is found to be safe and immunogenic we plan to progress to proof-of-concept Phase II efficacy studies.

37. *Molecular Methods Reveal Extensive Subpatent Infections in Pilot Epidemiology Study in India*

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Malaria is a serious global public health problem and the cause of high morbidity and mortality in the Indian subcontinent. *Plasmodium vivax* and *Plasmodium falciparum* are the predominant human malaria species in India although there have been reports of *P. malariae* detection in some regions. Primary diagnosis of malaria in India is mainly through passive case detection (PCD) via standard microscopy examination of blood smears collected from febrile patients. As part of the Center for the Study of Complex Malaria in India (a partnership between the National Institute of Malaria Research in India and New York University) we conducted a Pilot Epidemiological Survey via PCD at three study sites representing varied ecological niches to: (i) compare the sensitivity and specificity of three diagnostic tools [microscopy, rapid diagnostic test (RDT)] and species-specific diagnostic PCR), (ii) select the most suitable RDT device for use in the field, and (iii) examine any shifts in the epidemiologic profiles within these regions that could be used to inform future epidemiological studies at the three sites. Data from this study show significant shifts in species dominance and high rates of mixed-species infections. Moreover, PCR diagnosis revealed a high rate of *P. vivax* subpatent parasitemia, underlying the limited accuracy of both microscopy and RDT. Due to this, the overall prevalence rate throughout these different ecological niches is estimated to be 20-60% higher than reported.

38. *Study of Antibody and Cellular Responses to* Plasmodium Vivax *Variant VIR Proteins During Pregnancy*

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VIR antigens may be considered as promising candidates for a *Plasmodium vivax* malaria vaccine. We set out to characterize naturally acquired cellular and antibody responses to VIR proteins in pregnant and non-pregnant women heavily exposed to malaria. This work is part of a multicenter cohort study (PregVax) conducted in pregnant women in five *P. vivax* endemic countries: Brazil, Colombia, Guatemala, India, and Papua New Guinea (PNG) funded by European Commission (under grant agreement FP7-HEALTH-201588). 2000 plasma samples collected during pregnancy and 200 plasmas collected after puerperium were included in this study. IgG levels against 5 recombinant proteins and two long synthetic peptides (PvLP1 and PvLP2) covering different VIR sequences were measured by

multiplex suspension array technology using the Bioplex platform. Cytokine production was assessed in peripheral blood mononuclear cells (PBMC) from 69 women from PNG after in vitro stimulation with the long peptides by flow cytometry and luminex. *P. vivax* infection was determined by microscopy and polymerase chain reaction (PCR). Antibody responses were detected to all VIR antigens tested, with the highest responses found in PNG. The lowest levels of antibodies were found at delivery, when comparing with mid-pregnancy or post-partum. PvLP1 and PvLP2 stimulated cytokine production in PBMCs from malaria-exposed women. PBMCs from women with a *P. vivax* PCR positive infection secreted significantly more IL-10, IL-2, MCP-1, G-CSF and IFN-α in response to PvLP2, and had a significantly lower percentage of $CD4^+$ and $CD8^+$ T cells producing IFN-γ than non-infected women.

39. *A New Vaccine and a New Challenge:* Plasmodium Vivax *Pre-Erythrocytic Vaccines Using MVA and Chimpanzee Adenovirus; The Value of Novel Transgenic Parasites to Assess Vaccine Efficacy*

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Two leading malaria vaccine candidates target the pre-erythrocytic stage in the mammalian host: RTS,S and recombinant MVA and chimpanzee adenoviruses (ChAds), both using *P. falciparum* antigens. All have been shown to be immunogenic, safe and protective in clinical trials. We have developed new *P. vivax* vaccine candidates using MVA and ChAd63 recombinant viruses expressing the pre-erythrocytic antigens CSP and TRAP from *P. vivax*. We present our results using TRAP. Immunisation with the *P. vivax* TRAP vaccine resulted in high titers of antibodies and frequencies of antigen-specific T cells in three mouse strains tested. Immunodominant T-cell epitopes were additionally mapped on the protein structure and all were specific to the vWA extracellular domain regardless of the mouse model. Importantly, protective ability of the vaccines was assessed using a newly developed transgenic *P. berghei* parasite expressing *P. vivax* TRAP, which permitted a further analysis of the mechanisms responsible for protection. Depletion of T cells with monoclonal antibodies indicated that protection relied on both $CD8^+$ T cells and antibodies against TRAP. Our data show that the recombinant viruses ChAd63 and MVA expressing PvTRAP are good pre-erythrocytic stage vaccine candidates with potential for future clinical application, while transgenic parasites expressing *P. vivax* antigens are a good option for early development of new vaccine candidates for this disease.

40. Plasmodium vivax *Infection Dynamics in a Cohort of Children from Papau New Guinea*

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In endemic settings it is generally unclear what proportion of blood stage infections arise from relapses rather than primary infections. We present an analysis of the infection dynamics in a cohort of 264 children in Ilaita, Maprik District, Papua New Guinea, where transmission is intense, most individuals harbor multiple infections, but they have also acquired some immunity. The cohort, aged one to three years at enrolment, were followed up over 16 months with nine routine time-points every two months. Blood samples were taken at the routine timepoints and at other times if the child was ill. Samples positive by microscopy or LDR were genotyped using high-resolution capillary electrophoresis for two *Plasmodium vivax* markers (*msp1*F3 and *MS16*) and *P. falciparum msp2*. The genotypes were summarized as longitudinal patterns of success or failure to detect a genotype over the routine timepoints (*e.g.,* 001000001). We fit the frequencies of these patterns to a model with primary infection, relapse, clearance and detectibility. We also consider different levels of recombination. We take treatment into account and fit the model separately for three age groups. We assume that the seasonality of *P. vivax*

primary infections follows that of *P. falciparum* since they are transmitted by the same vectors. The estimates suggest that relapses contribute a substantial proportion of blood stage infections.

41. *Anti-Relapse Treatment and Potential Elimination of* Plasmodium vivax *Malaria: Insights from a Transmission Model and Surveillance Data from Northwest India*

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Despite over a hundred million annual infections worldwide and increasing severity, *Plasmodium vivax* malaria remains largely a neglected disease. In particular, the dependence of this malaria species on relapses, and the public health implications of clinically targeting the dormant stage, are not well understood. To quantify relapse parameters and assess the population-level consequences of anti-relapse treatment, we formulated a transmission model for *P. vivax* suitable for parameter inference with a recently developed statistical method based on routine surveillance data. A low-endemic region in NW India, whose strong seasonality demarcates the transmission and relapsing seasons, provides an opportunity to apply this modeling approach. Our model gives maximum likelihood estimates of 7.1 months for the mean latency and 31% for the relapse rate, in close agreement with regression estimates and clinical evaluation studies in the area. With a baseline of current treatment practices, the model predicts that an effective anti-relapse treatment of 65% of the infected population would eliminate the disease within a decade, and that periodic mass treatment would dramatically reduce the disease burden in a few years. The striking dependence of *P. vivax* on relapses for survival reinforces the urgency to develop a more effective anti-relapse therapy to replace Primaquine, the only available drug for the last fifty years. Our methods can provide alternative and simple means of estimating relapse parameters using routine epidemiological data, and evaluating the population-wide impact of relapse treatment in similar regions.

42. *A* Plasmodium vivax *Cross for Genetic Analysis of Chloroquine Response*

Juliana M. Sa, MSc, PhD[1], Sarah R. Kaslow, BA[1], Jiangbing Mu, MD[1], Rebecca Salzman, BA[1], Roberto M. Barros, PhD[1], Viviana Melendez-Muniz, BS[1], Evan G. Kessler, BS[1], Soundarapandian Velmurugan, PhD[2], Yonas F. Abebe, MSc[2], Eric R. James, MSc, PhD[2], Adam Richman, PhD[2], Sumana Chakravarty, PhD[2], Robert W. Gwadz, PhD[1], B. Kim Lee Sim, MSc, PhD[2], Stephen L. Hoffman, MD[2], Thomas E. Wellems, MD, PhD[1]; [1]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, Maryland, United States, [2]Sanaria Inc., Rockville, Maryland, United States

The genetic basis of *Plasmodium vivax* chloroquine resistance is unknown. Laboratory investigations rely on parasite strains adapted to non-human primates as *in vitro* investigations are limited by the lack of effective cultivation methods. To identify *P. vivax* genes possibly involved in chloroquine resistance we generated a genetic cross between two lines with distinct levels of tolerance to chloroquine. After producing a cross of these lines in *Anopheles* mosquitoes and a *Pan troglodytes*, we collected mixed pools of progeny and used *Aotus nancymae* and *Saimiri boliviensis* monkeys to evaluate *P. vivax* chloroquine response. Linkage group selection analysis identified a major chromosome region near *pvcrt*, a homolog of the *P. falciparum* chloroquine resistance transporter gene. Analysis of candidate genes and deep sequencing of the progeny before and after chloroquine selection are underway to refine mapping.

43. *Strengthening Malaria Control and Elimination in the Asia-Pacific*

Lorenz von Seidlein, PhD, MSc, The APMEN *Vivax* Working Group Coordinating Team[1]; [1]Menzies School of Health Research and Charles Darwin University, Darwin, Australia

The last decade has witnessed substantial success of malaria control efforts, with significant reductions in the incidence of *Plasmodium falciparum* malaria across many parts of the Asia-Pacific region. As malaria control efforts intensify, the relative proportion of *P. vivax* tends to rise reflecting the greater challenge for the control and elimination of this parasite. The Asia-Pacific Malaria Elimination Network (APMEN) was formed in 2009 to support national efforts towards regional elimination of malaria. APMEN's *Vivax* Working Group (VxWG) has representatives from the national malaria control programs of all 14 member countries and key research partners. A team based at the Menzies School of Health Research in Darwin, Australia coordinates activities, with support from the APMEN Secretariat based at the University of Queensland. The VxWG meets regularly, providing a unique forum to discuss, prioritise and facilitate research activities in the region. Action plans have been developed on several core themes including the conduct of anti-relapse clinical trials, parasite surveillance, G6PD diagnostics and parasite genotyping. Funding has been provided to member countries through a research grants program, which to date have been awarded to 22 recipients from nine countries in the region. The VxWG has also organized regular technical workshops, bringing together expert researchers in the field alongside country partners to foster consensus on regional priorities, collaboration on scientific protocols, and to encourage sharing of clinical and laboratory data. These activities are building partnerships and collaborative agendas that will provide crucial evidence for rationalizing control efforts as we move towards malaria elimination.

44. *Infectious Diseases that May Awaken the Hypnozoites of* Plasmodium vivax *Malaria*

G. Dennis Shanks MD[1], Nicholas White FRS[2]; [1]Australian Army Malaria Institute, Enoggera, Australia; [2]Mahidol Oxford Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

The periodicity of *Plasmodium vivax* malaria relapses may be explained by activation of latent hypnozoites initiated by preceding malaria and possibly other febrile infectious diseases. Historical records were reviewed to examine temporal relationships between *Plasmodium vivax* relapses and preceding febrile infectious diseases. In British soldiers in Palestine in 1918, epidemic falciparum malaria triggered a smaller epidemic of *P. vivax* relapses only in those who had previously been exposed extensively to malaria. Relapses did not follow pandemic influenza in soldiers known to have *P. vivax* following weeks of quinine administration. Typhoid fever may also activate *P. vivax* hypnozoites based on evidence from simultaneous typhoid and malaria epidemics in Puerto Rico and Guyana. Limited data suggest that febrile illness resulting from bloodstream infections caused by relapsing fever, trench fever, typhus and brucellosis may also provoke *P. vivax* relapse. Systemic parasitic and bacterial but not viral infections may activate *P. vivax* hypnozoites. Although falciparum recrudescences are common after trauma or severe stress, this is not true for *P. vivax* relapses. This suggests that specific components of the host's acute febrile inflammatory response and not fever alone are important factor(s) provoking relapse of *P. vivax* malaria. Future trials in animal systems using specific inflammatory activators may be informative.

45. *A Call to Arms: On Refining* Plasmodium vivax *Microsatellite Marker Panels for Comparing Global Diversity*

Patrick L. Sutton, PhD[1]; [1]New York University, Center for Genomics and Systems Biology

Microsatellite (MS) markers have become an important tool for studying the population diversity, evolutionary history, and multiplicity of infection in eukaryotic parasites. For the malaria parasite *Plasmodium vivax*, the utility of these microsatellites may even extend to describing infection dynamics across time, with respect to relapses, recrudescent infections, reinfections, and/or new infections. Over the past decade, approximately 240 MS markers have been published in the literature, yielding nine different panels of markers. However, inconsistent usage of each panel has resulted in a surfeit of descriptive genetic diversity data that remains largely incomparable between global populations. The

objective of this study was to statistically evaluate the quality of published *P. vivax* MS markers and devise a refined panel of MS markers to facilitate comparative genetics/genomics of extant populations. Utilizing the population metrics, genetic diversity indices, and MS parameters from 22 global *P. vivax* population diversity studies, robust statistical analyses were performed to differentiate between high and low quality MS markers. A statistically validated panel was formed, which includes 10 high quality MS markers scattered across 10 chromosomes. This refined panel reliably detects and describes global diversity as a function of endemicity, while accommodating the polymorphic potential of each MS marker.

46. *Improving the Safety and Efficiency of 8-Aminoquinolines for Treatment and Radical Cure of* Plasmodium vivax *Malaria*

Babu L. Tekwani, PhD[1], N.P. Dhammika Nanayakkara, PhD[1], Rosemary Rochford, PhD[2], Victor Melendez, PhD[3], Gregory Reichard, PhD[3], Brandon Pybus, PhD[3], Colin Ohrt, MD[3], Larry A. Walker, PhD[1]; [1]National Center for Natural Products Research, University of Mississippi, University, Mississippi, United States, [2]Department of Microbiology and Immunology, State University of New York Upstate, Syracuse, New York, United States,[3]Department of Experimental Therapeutics, Walter Reed Army Institute of Research, Silver Spring, Maryland, United States

Most malaria research and antimalarial drug discovery programs have emphasized malignant *Plasmodium falciparum* malaria as compared to "benign" *Plasmodium vivax* malaria, due to the high mortality. *P. vivax,* responsible for 70–80 million clinical cases per annum outside of Africa, also cause profound anemia, other severe symptoms and even death, as reported recently. Development of dormant hypnozoites in the hepatic tissues, cause malaria relapse and poses the major challenge for treatment of *P. vivax* malaria. The 8-aminoquinoline (8-AQ) antimalarials are active against *P. vivax* hypnozoites and stage five *falciparum* gametocytes. Drugs that target these hard-to-kill parasite stages are critical for malaria control/elimination. However, utility of this class has been limited because subjects with G6PD deficiency develop hemolytic anemia after treatments with 8-AQs. A number of new analogs have been synthesized and basic SARs have been established for improving therapeutic index of 8AQs. Until now, it has been difficult to select a member of this class that does not cause hemolysis, primarily due to lack of predictive preclinical models. Our recent work suggests that the toxicity of the currently available 8-AQs can be reduced by modification of the reactivity of the metabolically labile sites, and by separation of the racemic mixtures of current drugs into their specific enantiomers. An *in vitro* test and an animal model for human G6PD deficiency have been developed as tools for predicting the human hemolytic toxicity. Application of new and existing models and new knowledge on mechanism of toxicity of 8-AQs, have generated unprecedented opportunities for o ptimizing efficacy and improving safety of the 8-AQs for treatment of *P. vivax* malaria.

47. *Update on Development of NPC1161B, a Single Enantiomer 8-Aminoquinoline with Potential as a Safer Treatment and Radical Cure of* Plasmodium vivax *Malaria: Status and Prospects*

Larry A. Walker, PhD[1], Babu L. Tekwani, PhD[1], Mahmoud Elsohly, PhD[2], Ikhlas A. Khan, PhD[1], Leo Pavliv, RPh[3], Diane Keeney, PhD[2], Rosemary Rochford, PhD[4] and N.P. Dhammika Nanayakkara, PhD[1]; [1]National Center for Natural Products Research, University of Mississippi, University, Mississippi, United States, [2]Elsohly Laboratories Incorporated, Oxford, Mississippi, United States, [3]Cumberland Experimental Therapeutics, Nashville, Tennessee, United States, [4]State University of New York Upstate, Syracuse, New York, United States

Most of the antimalarials currently in clinical use are chiral but are still being used as racemates. In several cases, significant stereoselectivity has been noted in their pharmacological, pharmacokinetic and safety profiles. Recent studies in our lab have shown that enantiomers of primaquine have different metabolism and toxicity profiles, and that a newer analog, N4-(5-(3,4-dichlorophenoxy)-6-methoxy-4-methylquinolin-8-yl)pentane-1,4-diamine succinate (NPC1161C), has an even more dramatic enantioselectivity profile. However, the ultimate impacts of this for potency and safety of the drugs in man are still not clear. The racemic NPC1161C, originally prepared by WRAIR (as WR233078), shows excellent *in vivo* oral activity against blood & tissue stages of malaria parasite. NPC1161C was resolved

into two enantiomers namely NPC1161A, the (+)(*S*) enantiomer and NPC1161B, the (-)(*R*) enantiomer. A cost-effective process for obtaining enantiomerically pure preparations of NPC1161B has been optimized. Though both the enantiomers show good oral efficacy at very low doses in mouse models of malaria, NPC1161B is 5-20 times more potent than NPC1161A. Interestingly NPC1161B caused markedly reduced methemoglobinemia in Beagle dogs and significantly reduced systemic toxicity in rodents as compared to NPC1161A. Comparing the metabolic pathways, it appears that different CYP isoforms mediate the metabolism of NPC1161A and B, and this may account for the reduced toxicity. In *Plasmodium cynomolgi*-primate model, the only reliable model to test antihypnozoite potential, excellent efficacy of NPC1161B for radical cure was established with a 3-day regimen at sub-mg daily doses. Preclinical regulatory safety studies have been completed. The status and plans with regard to clinical development will be discussed.

48. *Identification of a Potent non-8-Aminoquinoline Compound that Kills* Plasmodium cynomolgi *Dormant Liver Stage Parasites* In Vitro

Anne-Marie Zeeman, PhD[1], Sandra van Amsterdam, MSc[1], Case McNamara, PhD[2],Els Klooster, BSc[1], Nicole van der Werff, MSc[1], Dominique Mazier, MD, PhD[3], Brice Campo, PhD[4], Didier Leroy, PhD[4], Robert Sauerwein, MD PhD[5], Elizabeth Winzeler PhD[2], Bryan Yeung, PhD[6], Clemens Kocken, PhD[1]; [1]BPRC, Rijswijk, the Netherlands, [2]Genomics Institute of the Novartis Research Foundation, San Diego, California, United States, [3]Institut national de la santé et de la recherche médicale, Paris, France, [4]Medicines for Malaria Venture, Geneva, Switzerland, [5]Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands[6]Novartis Institute for Tropical Diseases, Singapore

Of all known malaria parasites, *Plasmodium cynomolgi* has the highest genetic and biological similarity with *P. vivax*. *P. cynomolgi* in rhesus monkeys has historically been used to identify compounds with anti-hypnozoite activity and this resulted in primaquine (PQ), currently the only anti-relapse drug in clinical use. We have recently described an *in vitro* liver stage culture system using *P. cynomolgi* (Dembele *et al.* 2011, PloSOne 6(3):e18162), in which small persistent liver stage parasites were observed that strongly resemble hypnozoites. We exploited this robust liver stage culture platform to screen new compounds for activity against hypnozoite-forms. We initially screened a series of 18 known antimalarials and 14 new non 8-aminoquinolines (preselected for blood and/or liver stage activity) in three-point tenfold dilutions (0.1, 1 and 10 µM end concentration). A novel compound showed a profile similar to PQ, efficiently killing both *in vitro* cultured developing liver stages and hypnozoite-forms (IC_{50} for hypnozoite-forms: 0.69 µM and PQ: 0.84 µM, for developing liver stages: 0.64 µM and PQ 0.37 µM). When given as causal prophylaxis in a rodent malaria model, a single oral dose of 100 mg/kg prevented blood stage parasitemia. From these results we conclude that this new compound may represent a new class for radical cure of *P. vivax* malaria.

Poster Session 2
Wednesday, May 29, 2013
1:00 PM – 2:30 PM

1. Camila Bôtto-Menezes
2. Alexander Franco-Gallego
3. Shilpi Garg
4. Najia K. Ghanchi
5. Najia K. Ghanchi
6. Lilia Gonzalez-Ceron
7. Sonal Gupta
8. Muzamil M. Abdel Hamid
9. Flora S. Kano
10. Flora S. Kano
11. Johanna H. Kattenberg
12. Anju Kochar
13. Dhanpat K. Kochar
14. Sanjay K. Kochar
15. Kailash Kothia
16. Karina Leiva
17. Luciana C. Lima
18. Stefanie C.P. Lopes
19. Gisely Melo
20. Vitor R.R. Mendonça
21. Sheetal Middha
22. Cassian Mwatele
23. Wang Nguitragool
24. Bakri Y.M. Nour
25. Rintis Noviyanti
26. Rintis Noviyanti
27. Shashiraja Padukone
28. Rapatbhorn Patrapuvich
29. Paulo Pimenta
30. Surendra K. Prajapati
31. Afsheen Raza
32. Afsheen Raza
33. Claudia M. Ríos-Velásquez
34. Leanne J. Robinson
35. Mauricio M. Rodrigues
36. Wanlapa Roobsoong
37. Catalina Saavedra
38. Meddly L. Santolalla
39. Ari Satyagraha
40. Vishal Saxena
41. Nágila Secundino
42. Sneh Shalini
43. Hemanand SP
44. Dewi Susanna
45. Gajanand Singh Tanwar
46. Priya Tanwar
47. Katherine Torres

1. ***Malaria in Brazilian Pregnant Women***

Camila Bôtto-Menezes[1,2], Azucena Bardají[3], Flor E. Martínez-Espinosa[1,4] ; [1]Fundação de Medicina Tropical Dr. Heitor Vieira Dourado, Manaus, Amazonas, Brazil, [2]Universidade do Estado do Amazonas, Manaus, Brazil, [3]Barcelona Centre for International Health Research, Hospital Clinic, University of Barcelona, Barcelona, Spain [4] Centro de Pesquisas Leônidas e Maria Deane, Fiocruz Amazônia, Manaus, Amazonas, Brazil

Plasmodium vivax is the most prevalent malaria species in the American region, and responsible for more than 80% of the malaria cases. Brazil accounts for the higher number of the malaria cases reported in pregnant women in the Americas. This study aims to describe the epidemiology of clinical malaria during pregnancy, and the frequency and risk factors associated with prematurity and low birth weight in pregnant women that attended the FMT-HDV in the Amazon region, between December 2005 and March 2008. A total of 503 pregnant women with clinical malaria were included in this analysis. Most of them were young (82%), reported that had more than one clinical malaria episode during pregnancy (36%) and were found to be anemic (59%). The prevalence of low birth weight (9%) was similar to that observed in general population (8.1%); however the prevalence of premature delivery (16%) showed more than two-fold increase compared to general population (6.6%). Younger women, primigravidae and those with less access to antenatal care showed an increased risk of prematurity and low birth weight. The results of this study reveals that *P.vivax* is becoming the predominant species among women with clinical malaria during pregnancy, and that *P.vivax* malaria is an important public health problem with harmful consequences for the health of the woman and their offsprings in this region.

2. ***Immune Response and Tissue Damage in Placental Malaria by*** **Plasmodium vivax** ***and Genotyping of vir E Genes in Clinical Isolates from Infected Pregnant and non-Pregnant Patients from Colombia***

Alexander Franco-Gallego, Microbiol[1], Olga Agudelo-García, Microbiol; MSc[1], Beatriz Aristizabal, Bacter; PhD[2], Stephanie Yanow, BSc; PhD[3], Sedami Gnidehou, BSc; PhD[3], Amanda Maestre, MD; PhD[1], Jaime Carmona-Fonseca, MD; MSc[1]; [1]Grupo de Investigación Salud y Comunidad, Universidad de Antioquia, Medellín, Colombia, [2]Hospital Pablo Tobón Uribe, Medellín, Colombia, [3]Provintial Laboratory for Public Health, Edmonton, Alberta, Canada

Placental changes associated with malaria include hypoxia and cellular infiltrates that regulate cytokine profiles, and disrupt the immune balance of the placenta required for a successful pregnancy. Some reports show that *Plasmodium vivax* exhibits cytoadherence as a mechanism of pathophysiology related with vir genes. Aimed at establishing the relationship between placental *P. vivax* infection with tissue damage, production of Th1/Th2 cytokines and vir E genotyping in different populations, a study was carried out in Northwest Colombia. Samples were obtained from 30 pregnant, 39 non-pregnant patients and 10 placentas *P. vivax* positive by RT-PCR; 10 negative placentas were included as a controls. The expression of inflammation and hypoxia markers (HIF-1, VEGF, COX-2 and COX 1) and cytokines (IL-2, IL-4 , IL-10, TNF-α, IFN-γ) were determined by RT-PCR. Apoptosis was detected by DeadEndTM Colorimetric TUNEL System. For genotyping of vir E genes, a PCR with degenerated primers was performed followed by cloning and sequencing. The results confirm the predominance of pro-inflammatory cytokines and significant increase in inflammation, which is determined by the expression of COX-1 and COX-2 genes in infected placentas. We also observed a significant increase in preapoptosis and apotosis in placental tissue. *P. vivax* genotyping for vir E genes in each population, confirmed differential distribution in three groups. Placental infection by *P. vivax* in the studied population evidences the deleterious effect on this species in placental tissue.

3. *Novel Point Mutations in the Antifolate Drug Resistance Marker Genes among* Plasmodium vivax *Isolates Exhibiting Severe Manifestations*

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Plasmodium vivax is one of the predominant species of the human malaria parasite present in the Indian subcontinent. There have been recent reports on Chloroquine resistance and severe manifestations shown by *P. vivax* from different regions of the world including India. This study focuses on Bikaner, India where during the last few years there have been continuous reports of severe manifestations by both *P. falciparum* and *P. vivax*. Chloroquine and Sulfadoxine-Pyrimethamine have a widespread use for treating malaria in this region. We report here the profile of mutations in marker genes associated with resistance to these drugs among the *P. vivax* parasites obtained from patients with severe and non-severe manifestations. Most isolates showed the wild type alleles for both of these markers ($P<0.0005$). The frequency of PvDHFR-PvDHPS two locus mutations was higher among the patients showing severe manifestations ($P<0.003$). Novel mutations in PvDHFR and PvDHPS were observed only in the parasite population from patients exhibiting severe complications. Preliminary homology modeling and molecular docking studies predicted these mutations not to have any effect on binding of the drug molecule to the enzyme. However, the presence of novel mutations in the PvDHPS gene indicate polymorphic nature of this molecule which is in contrast to available published information. For few of the above samples, genotyping was performed using well established markers like MSP-3α and CSP. We have also studied the expression pattern of these genes in severe malaria cases. The microarray expression data showed any one of the genes; Pvcrt or Pvmdr-1 to be upregulated. Recent reports have also suggested an increased expression of the chloroquine resistance marker genes in severe *vivax* malaria. These findings supports to further explore potential of *these genes as* molecular markers of severe disease in *P. vivax*.

4. *Genetic Diversity of* Plasmodium vivax *and* Plasmodium falciparum *Clinical Isolates from Southern Pakistan*

Najia K. Ghanchi, PhD[1], Afsheen Raza, MSc[1], Ali M. Thaver, MBBS[2], Sana Jafri, MSc[1], Mohammad A. Beg, PhD[1]; [1]Aga Khan University, Karachi, Pakistan, [2]Aga Khan Medical College, Karachi, Pakistan

Plasmodium vivax and *P. falciparum* co-exist in Pakistan with *P. vivax* being the prevalent specie accounting for 70% of malaria burden; however, there is no baseline data available on circulating genotypes. Prevalent genotypes of *P. falciparum* and *P. vivax* with an aim to bridge the existing knowledge gap on population structure of malaria from Pakistan. During January 2006 to May 2009, a total of 250 and 244 blood samples were collected from patients tested slide positive for *P. vivax* and *P. falciparum* mono-infections respectively. Nested PCR/RFLP was performed, using pvcsp, pvmsp1and pfmsp1 & 2 markers to detect the extent of genetic diversity in clinical isolates of *P. vivax* and *P. falciparum* from Southern Pakistan. A total of 227/250 (91%) isolates were included in the analysis of *P. vivax* and 238/244 (98%) of *P. falciparum.* In pvmsp1, a total of 87 genotypes were detected while in pvcsp, both VK 210 (85.5%, 194/227) and VK 247 type (14.5%, 33/227) were found to be circulating in *P. vivax* isolates from Southern Pakistan. Whereas, only 56/231 (24%) and 51/236 (22%) carried multiple *P. falciparum* genotypes in msp-1 and msp-2, respectively. We have observed limited diversity in pfmsp 1 and pfmsp 2 genes of *P. falciparum* isolates compared to high diversity in pvcs and pvmsp1 genes of *P. vivax***.** This study confirms that extensively diverse pvcsp and pvmsp1 variants of *P. vivax* are circulating within this region. Results from this study provide valuable data on genetic diversity of *P. vivax* which will be helpful for further studies and development of CSP and MSP-1 based vaccines against *P. vivax*.

5. *Chloroquine-Resistant* Plasmodium vivax*: An Emerging Threat from Pakistan*

Najia K. Ghanchi, PhD[1], Afsheen Raza, MSc[1], Syed Faisal Mahmood, MBBS[1], Muhammad Junaid Patel, MBBS[1], M. Asim Beg, MBBS, PhD, FRCP(Edin)[1]; [1]Aga Khan University, Karachi, Pakistan

Emerging resistance to chloroquine (CQ) in *Plasmodium vivax* poses huge burden on health of millions of people exposed to the risk of *vivax* malaria. CQ is the recommended anti-malarial drug for treatment of *P. vivax* infection. However, reports of CQ resistance are consistently being documented worldwide. In recent year number of sever malaria cases are increasingly reported from Pakistan. In this study, two *in vivo* CQ resistant cases of *P. vivax* presented at Aga Khan University Hospital, Karachi were analyzed for molecular markers of drug resistance. Blood samples from patients were microscopically confirmed for *P. vivax* mono-infection. DNA was extracted from patient blood samples and amplified using PCR/RFLP for genotyping and drug resistance associated markers pvmsp-1, pvcsp, pvdhfr, pvdhps and pvcrt-0. Genotyping analysis revealed that the samples carried pvmsp-1 Type 1 and pvcsp VK 210 repeat types. Furthermore, analysis of sulphadoxine-pyrimethamine (SP) resistance associated mutations detected presence of 117N, 50I and 119K mutations ;both 117N and 50I mutation have been associated with emerging resistance against SP implying that both patients were infected with SP resistant strain of *P. vivax*. Interestingly, no mutation was observed in the pvcrt-o gene.

This is the first report of *P. vivax* Chloroquine resistance malaria in Pakistan. Molecular markers along with In vitro susceptibility testing of *P. vivax* may provide a useful tool to highlight areas of emerging chloroquine resistance. In conclusion, it is suggested that clinically treatment failure cases need to be analyzed with these tools so the extent and impact of drug pressure can be monitored effectively.

6. Polymorphism and Evolutionary Relationships of the *Plasmodium Vivax* Circumsporozoite Gene in Latin America

Lilia Gonzalez-Ceron, PhD[1], Jesus Martinez-Barnetche, PhD[1], Ciro Montero-Solís, PhD[1] Frida Santillán, Bsc[1], Aida M. Soto, MD[2] Mario H. Rodríguez, PhD[1] Benjamin J. Espinosa, PhD[3], Octavio A. Chávez, MD[4];[1]Instituto Nacional de Salud Pública, Mexico, [2]Pan American Health Organization Office, Managua, Nicaragua, [3]US Naval Medical Research Unit No. Six (NAMRU-6), Lima, Peru, [4]Vector Control Program, Silais Chinandega, Ministry of Health, Nicaragua

Although progress on *Plasmodium vivax* genetics and genomics has been achieved worldwide, more information on the genetic diversity in the Latin-America is needed to better explain current patterns of parasite dispersion and evolution. Circumsporozoite gene polymorphism was investigated using PCR-RFLP and DNA sequencing in isolates from the Pacific Ocean coast of Mexico, Nicaragua, and Peru. Four RFLP subtypes (vk210a, b, c and d) were identified in Mexico and three subtypes (vk210a, e and f) in Nicaragua. The nucleotide sequences showed that Mexican vk210a and all Nicaraguan isolates were similar to other American parasites and reflect their circulation throughout the continent. In contrast, vk210b, c and d were less frequent, had a domain ANKKAEDA in their carboxyl end and clustered with Asian isolates, first detected in Latin America. All vk247 isolates from Mexico and Peru were identical. Their genetic relationships and low variability within vk210b, c, d and/or within the vk247 parasites in southern Mexico suggest its recent introduction to this region. Differences in mismatch distribution parameters of the central repeat (CR) separate vk247 from most vk210 isolates; while vk247 isolates display a homogeneous pattern with no geographical clustering, vk210 isolates display a heterogeneous geographically clustered pattern and differences in the CR allelic type frequencies which clearly separates Latin-American (vk210 a, e and f) from non-American isolates. The global analysis of *P. vivax* csp suggests this parasite migration to Latin America in at least two different time-points likely affected by bottlenecks and dispersal events in the Americas.

7. *Identification of Minimal Binding Domain of* Plasmodium vivax *Reticulocyte Binding Protein*

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Plasmodium vivax preferentially invades reticulocytes which comprise ~1 % of red cells in normal human blood. This specificity was demonstrated to be associated with two apical merozoite proteins namely RBP1 and RBP2 (reticulocyte binding proteins 1&2). We have mapped the reticulocyte binding domain (RBD) of RBP1 near the N-terminal region of PvRBP1. This region was chosen on the basis of sequence homology with erythrocyte binding regions of *P. falciparum* reticulocyte binding protein homologues (RH proteins). This 248 amino acid region expressed on the surface of COS7 cells showed specific binding to reticulocytes. Binding of transfected COS7 cells with reticulocytes was assessed by rosette formation in the binding assay. Further, this region was produced as recombinant protein in soluble form in E. coli. The 30kDa recombinant protein (rRBP1-RBD) was found to bind reticulocytes with specificity. Antibodies against rRBP1-RBD were shown to inhibit binding of transfected COS cells expressing RBP1-RBD to reticulocytes. Functional region of RBP1 may serve as a promising vaccine candidate for *P. vivax* malaria.

8. *Evidence for* Plasmodium vivax *Duffy-Independent Transmission from Sudan*

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Malaria is a significant public health concern in Sudan where approximately seven million cases are reported annually. Malaria infections in Sudan are mainly caused by *Plasmodium falciparum*. However, in recent years the number of *Plasmodium vivax* infections is increasing. Furthermore, severe cases due *P. vivax* were recently reported. In this study we investigated the frequency of the FYES allele and *P. vivax* infection in malaria patients from the central Sudan. Blood samples were collected from 300 individuals representing three sites. DNA was extracted from dried blood spots collected from malaria patients using Chelex method. Identification of the *Plasmodium* species was achieved by nested PCR amplification of the small-subunit rRNA genes; Positive *P. vivax*-human isolates (N=80) were genotyped for the Duffy blood group through the analysis of the DARC gene. PCR and restriction enzyme digestion were used to detect the presence of the FYES allele. Of all samples tested for the FYES allele, 10% (8/80) had the FYES allele. Results were confirmed by DNA sequencing. We provide first molecular evidence of Duffy-independent red cell invasion. The emergence of confirmed *P. vivax* infections in Sudan could be due to the fact that the parasite must be evolving alternative red cell invasion pathways.

9. *Duffy Antigen Receptor dor Chemokines (DARC) Polymorphisms and its Involvement in Inhibitory Anti-Duffy Binding Protein II (Dbp_{II}) Immunity*

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The *Plasmodium vivax* Duffy binding protein (PvDBP) and its erythrocytic receptor, the Duffy antigen receptor for chemokines (DARC), are involved in the major *P. vivax erythrocyte invasion* pathway. Here, in an agricultural settlement of the Brazilian Amazon area, we carried-out an open cohort study to analyzed DARC genotypes and its relationship to the PvDBP immune response. Among 690 individuals enrolled in the study, the distribution of DARC genotypes was consistent with the

heterogeneous ethnic origin of the Amazon population, with a predominance of no silent FY alleles: FY*A > FY*B. In the study area, no association was found between DARC genotypes and *P. vivax* susceptibility. Of importance, the follow-up study demonstrated that binding inhibitory antibodies targeting PvDBP (region II) towards to be more frequent in heterozygous carrying a DARC-silent allele ($FY*B^{ES}$). No association was found with antibodies to $PvMSP1_{19}$, another *P. vivax* protein, and DARC genotypes. Together, these results provide the first evidence that DARC polymorphisms may influence the naturally acquired inhibitory anti-Duffy Binding Protein II immunity.

10. *Atypical Memory B Cells in* Plasmodium vivax*-Exposed Individuals*

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Our previous studies suggested that antibodies against *Plasmodium vivax* are short-lived. Recently, a new subset of Memory B Cells (MBCs) was identified during *P. falciparum* infection, and these atypical MBCs might impair the generation of an effective memory response. Hence, our hypothesis is that the presence of these atypical MBCs in *P. vivax* infection explains the short-lived antibody response. Peripheral Blood Mononuclear Cells (PBMCs) were isolated from malaria exposed individuals with (i) acute *P. vivax* infection (N=23); (ii) previously exposed but currently uninfected individuals (non-acute, N=34); as a negative control we included; (iii) naïve individuals from a non-endemic area (N=14). Using flow cytometric, the frequency of atypical MBCs was significantly higher in subjects exposed to malaria (acute group: 10.63%, and non-acute group: 9.98%) compared to naïve subjects (3.88%). Furthermore, activated MBCs (2.30%) and plasma cells (2.30%) were also significantly higher in subjects with acute malaria than unexposed naïve group (0.98% and 1.17% for activated and plasma cells, respectively). However, we didn't find a significant difference in frequencies of conventional B cells (naïve, immature and classical) between malaria-exposed but currently uninfected and naïve individuals. Although the levels of anti-*P. vivax* antibodies to the DBP and $MSP1_{19}$ antigens didn't correlate with B cell response, atypical MBCs were more frequent in individuals who had not developed specific antibodies during the follow-up study (non-responders: 10.98%, and responders: 8.19%; p=0.0327). Altogether, these results suggest that atypical MBCs might compromise the generation of malarial antibody responses. Further studies will be required to define the role of atypical MBCs in malaria *P. vivax* infection.

11. *Erythrocyte Polymorphisms Associated with Protection against* Plasmodium vivax *Malaria in Papua New Guinean Children*

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According to the "malaria hypothesis", the high frequencies of deleterious mutations in some human populations are potentially caused by strong selective pressure of malaria. Populations of the South West Pacific, a co-endemic region for all four human malaria parasite species, are highly diverse and exhibit a range of unique red blood cell polymorphisms, with geographical patterns paralleling malaria-endemicity. In an earlier study we showed that South East Asian Ovalocytosis (SAO) (caused by band 3 deletion SLC4A1Δ27) was associated with a reduction in risk of *Plasmodium vivax* infections and clinical episodes and revealed the potential for a strong protection against severe *P. vivax* malaria,

thus demonstrating the potential selective pressure of *P. vivax* on the human genome in PNG. Here we now present results on the association of the Gerbich blood group variants (caused by a deletion in exon3 of the glycophorin C gene (GYPC) with risk of *P. vivax* and *P. falciparum* malaria in PNG children. In a cohort of children of 1-3 years from East Sepik, PNG, the GYPC homozygote genotype was associated with strong protection against *P. vivax* malaria that increases in strength with increasing parasitemia. In addition, we will present data on the associations of GYPC genotypes with burden of *P. vivax* infection and disease in two further cohorts of children 3-21 months and 1-4yrs from East Sepike and Madang provinces. Together, these data strongly suggest that *P. vivax* malaria may have contributed to shaping the unique host genetic adaptations to malaria in Asian and Pacific populations.

12. *Ocular Manifestation in Adult Patients of* Plasmodium vivax *Malaria*

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Plasmodium vivax malaria had been reported having severe manifestation as seen in *Plasmodium falciparum* malaria from different part of world. This also included occasional cases of ocular abnormalities including subconjunctival and retinal hemorrhage. This prospective study was done in 70 hospitalized cases of *P. vivax* malaria with varying severity to study the ocular changes associated with it. The diagnosis of malaria was done by peripheral blood film (PBF), rapid diagnostic test (RDT) and further confirmed by polymerase chain reaction (PCR) in selected cases. The patient was subjected to different clinical and biochemical tests to rule out associated co-morbid condition causing similar changes in eye. The ophthalmological findings were detected in 11 patients (17 eyes). Retinal vascular changes (mild to moderate arterial tortuousity and venous dilation) in five patients (10 eyes), blurring of disc margin were seen in two patients (three eyes), isolated hard exudate near equator in two patients (two eyes), and subconjunctival hemorrhages in two patients (two eyes). The retinal hemorrhages was present in two patients (three eyes). The first patient showed one large flame shaped hemorrhage near the disc and two pre-retinal hemorrhages, one overlying the fovea and other close to it which led to decreased vision (visual acuity – 6/24, J3) and central scotoma. Fundus photograph was taken and will be shown during presentation whereas the second had multiple Roth spots (>10) in each eye and large sub-hyaloid hemorrhage overlying macula in left eye. As patient was very sick, fundus photograph and visual field study could not be done. The details of other observations will be presented.

13. *Thrombocytopenia and* Plasmodium vivax *Malaria — Some Important Observations*

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Recent observation from Brazil, India and other countries had shown important association of severe thrombocytopenia (<20000/mm^3) and other severe manifestation. In a prospective study on admitted adult *Plasmodium vivax* patients 143/460(31.09%) had thrombocytopenia in which 26 patients (18.18%) had <20000/mm. The risk of developing thrombocytopenia was significantly more common in *P. vivax* comparison to *Plasmodium falciparum* malaria. However, the risk of severe thrombocytopenia was not statistically significant. Three patients had severe epistaxis and required platelets transfusion for management. In another prospective study on 380/676 admitted children with *P. vivax* monoinfection, thrombocytopenia was observed in 278/380 (73.16%), predominately in 0–5 age group. Severe thrombocytopenia was present in 60 Patients and the risk of developing was higher in *P. vivax* monoinfection in comparison to *P. falciparum* and mixed infection ($p<0.001$). Bleeding was present in 63/380(16.58%) children and was always associated with thrombocytopenia but the reverse was not always true. There was significant ($p<0.014$) association of severe malaria with severe

thrombocytopenia. In another recent prospective study on 546 patients of *P. vivax* monoinfection (age 1–60 years), thrombocytopenia was observed in 468 (85.71%) patients with a mean platelet count of 72715.8±38625.4 and mean parasite density of 6733.4±9523.8. The statistical analysis did not show any correlation between these two parameters (correlation co-efficient (R^2)=0.00. In PCR diagnosed *P. vivax* monoinfection patients. Conclusion: (1) thrombocytopenia including severe thrombocytopenia with or without bleeding is not uncommon. (2) There is strong association between severe thrombocytopenia and other severe manifestation in children. (3) There is no association between parasite density and platelet count.

14. *Hepatic Involvement in* Plasmodium vivax *Malaria*

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This prospective study was done on 1091 admitted patients of malaria (*Plasmodium falciparum:* 539, *P. vivax:* 473, mix: 79) during 2007 to 2008 to study the hepatic dysfunction in *P. vivax* malaria. The species diagnosis were done by peripheral blood film (PBF and/or rapid diagnostic test (RDT) and further confirmed by polymerase chain reaction (PCR) in all severe cases. Amongst the 473 cases of *P. vivax* malaria 221 fulfilled the WHO definition of severe malaria and 97 were having evidence of jaundice/hepatic dysfunction (serum bilirubin >3mg/dl). The mean serum bilirubin level was 6.32±4.79 (range 3.00–30.30) with predominantly conjugated hyperbilirubenimia. Serum bilirubin level of 3-5 mg/dl was present in 50; 5–10 mg/dl in 36 patients while more than 10 mg/dl was present in 11 patients. Mean AST level was 88-54 ± 105.36 (maximum 632.6 IU/l), mean ALT level was 140.02 ±186.8 (maximum 968.6 IU/l), mean alkaline phosphatase level was 153.65±142.28 (maximum 335.12 IU/l). All patients were studied for the markers for viral hepatitis along with ultrasonography of abdomen to rule out other concomitant illnesses. 17 patients had associated multi-organ dysfunction and the commonest was renal failure in 14 patients. Features of hepatic encephalopathy were detected in 8 cases. Six patients died and all of them had multi organ dysfunction (renal failure: 4, cerebral malaria: 4, anemia: 3, and thrombocytopenia: 1). Comparison with *P. falciparum* and mixed infection will be presented.

15. Plasmodium vivax *Malaria Death was Observed in Sentinels Surveillance Hospital Contained by Surat City, India*

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Malaria is one of the most widespread parasitic diseases in the world. Surat, malaria stands as one of the major public health problem. Since, 1988 Surat Municipal Corporation (SMC) has established its own urban malaria scheme to combat the problem of malaria. The most important component of malaria control programme, which has a direct bearing on prevention of mortality and reduction in the morbidity, in community. A system generated in SMC that daily report of outdoor patient and indoor patient from 39 urban health centres, 12 major private and government hospital data were collected in report of fever and malaria. Details of patient name address, on set of illness, history of movement, treatment taken from clinic etc. As of 2007 to 2011 total of 250 malaria deaths were reported in Surat city. Owing to which 31 malaria deaths were found as a result of *P. vivax* infection and 198 deaths were contributed by *P. falciparum* infection. However 11 deaths were originated for mix (*P. vivax and P. falciparum*) infection and rest of 10 deaths were found by reason of fever related. Among 250 death cases were revealed 182 (72.8%) for male and 68 (27.2%) for female. Studies pointed out high case fatality rate (44.4%), 111 out of 250 in the age group of 15–44 years. Based on hospitals available records of Surat city showed that 163 death cases have parasitemia density and co-morbidity. The essential pathologic features of severe malaria have cerebral malaria (1.23%), renal failure (31.3%), hepatic dysfunction (37.4%) and ARDS (31.3%). However severe anaemia (22.7%) and thrombocytopenia (66.9%) that causes bleeding diathesis is produced by haemolysis, reduced cell deformity of parasitized and no parasitized erythrocytes increased splenic clearance, reduction platelet survival, decreased platelet production, and increased splenic update of platelets and can be produced by *P. vivax* and *P. falciparum* infection. In addition to above complication the parasite density observed mainly in severe *vivax* malaria and *P. falciparum* malaria respectively showed 829-49831/ µl and 234-77818/µl. Based on the prospective scrutinized study indicated that, *P. vivax* infection is generally mild and does not cause much mortality to the patient however, our study clearly indicated from the above findings that *P. vivax* infection can cause significant severe morbidity as well as the mortality.

16. *Serum Cytokine Responses Associated with Severe* Plasmodium vivax *Malaria in a Low Malaria-Endemic Area in the Peruvian Amazon Basin*

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Plasmodium vivax is the most widely distributed cause of malaria worldwide. Several reports from Asia and Brazil now indicate that *P. vivax* may cause severe malaria and deaths. However, the presence of severe *P. vivax* malaria in low endemic settings has not been well documented. In addition, the immune mechanisms associated with this process are poorly understood. To study the immune profile associated with severe presentations caused by *P. vivax* monoinfection, we conducted a case-control study in the Peruvian Amazon. Individuals with severe *P. vivax* malaria (cases) and uncomplicated malaria (controls) were enrolled from two major hospitals in Iquitos. Severe malaria was classified based on modified WHO criteria. Plasma samples were collected for cytokine analysis using Luminex xMAP technology. From March 2012 to January 2013, we enrolled 41 cases and 49 controls. Parasitemia counts were significantly higher in cases compared to controls. In addition, cases had increased levels of Interleukin (IL)-1β, IL-6, IL-10, IL-12, IL-13, IL-17, granulocyte macrophage colony-stimulating factor, gamma-interferon, monocyte chemotactic protein-1 and TNF-alpha in their plasma when compared to controls ($p<0.05$). Additionally, parasitemia positively correlated with all tested cytokines, indicating that the inflammation may be driven by parasite antigens. In conclusion, severe

malaria is common even in low malaria-endemic settings and it is associated with higher parasitemia and a strong inflammatory profile. We are currently assessing other inflammatory markers including superoxide dismutase and markers of endothelial cell activation

17. *Immunogenicity of Pichia Pastoris Recombinant Proteins Representing* Plasmodium vivax *Immunodominant Antigens from Different Parasite Stages*

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Considering the high complexity of malaria parasite life cycle, we hypothesized that an effective vaccine formulation against *Plasmodium vivax* malaria should contain multiple immunodominant antigens from different parasite stages. Based on that we studied the immunogenicity of a vaccine formulation consisting of recombinant proteins representing a mixture of a chimeric Circumsporozoite Protein (CSAll) which merges B cell epitopes of the allelic variants (VK210, VK247 and *vivax*-like) and the Apical Membrane Antigen 1 (AMA-1). The proteins were expressed in yeast Pichia pastoris GS115 strain and purified by two chromatography steps allowing a final product with high yield and purity, confirmed by RP-HPLC. C57BL/6 and BALB/c mice were immunized with the proteins, alone or in combination, in the presence of Poly(I:C) adjuvant, and induced immune response was assessed. IgG titers were determined by ELISA against the homologous proteins and the three PvCS variants. In C57BL/6, the seroconversion occurred after one CSAll dose, and after the 3rd the specific IgG raised didn't alter by combination with AMA-1. However in BALB/c, the CSAll response required two doses and IgG were impaired with combination. Interestingly, all PvCS variants were recognized. Cellular response was measured by T cell proliferation index in C57BL/6. A discrete $TCD8^+$ proliferation was verified with AMA-1 (<4%) and PvCS variants (ranging from 2–5%) stimulus, on groups immunized with alone proteins, but it was impaired on combination. This study demonstrates that the vaccination with the combination of antigens elicits potent antibody response in C57BL/6 mice, which recognizes the three PvCS allelic variants.

18. Plasmodium vivax*: Disproportion of Peripheral Circulating Schizonts and its Adhesive Capacity*

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It has been assumed for a long time that *Plasmodium vivax* infected erythrocytes (Pv-iEs) are not able to cytoadhere, as all stage-forms of this parasite are seen in patient's peripheral blood. Nevertheless, the 18th century literature have discussed about a small proportion of schizonts in relation to other parasitic stages in the peripheral blood. Moreover, two recent and independent findings showed the ability of Pv-iEs cytoadhere under static and flow conditions to lung and brain endothelial cells and to placenta cryosections. These observations challenge the current view regarding the inability of Pv-iEs to sequestrate in the microvasculature of several organs. Herein, we assessed the frequency and proportion of schizonts in the peripheral blood of 50 *P. vivax*-infected patients attended at the Manaus Tropical Medicine Hospital Foundation. Furthermore, we investigated the ability of *P. vivax* schizonts to cytoadhere to endothelial cells by cultivating Pv-iEs for 18-22h to maturation. Of 50 patients included in the study, 27 had no schizonts in circulation at the moment of blood harvested. Important, for those individuals who presented schizonts in peripheral circulation, a high disproportion of this stage-form in relation to other asexual stages was observed. Also, adhesion assays performed with Pv-iEs before or after ex-vivo maturation showed that mature parasites display a potent (6-fold) binding ability in comparison to non-matured stage-forms, and a positive correlation between schizonts percentage and cytoadhesion rate were also found. Collectively, these finding provide valuable insights on *P. vivax* biology.

19. *Expression of pvcrt-o and pvmdr-1 Genes Is Increased in Patients with Chloroquine Resistance and Complicated* Plasmodium vivax *Malaria*

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Mechanisms underlying severe malaria triggered by *Plasmodium vivax* have been poorly appreciated, mainly the increasing chloroquine-resistance, which has been implicated in the more severe disease burden. Different groups of patients were included in this study performed in the Brazilian Amazon: 1) patients with *P. vivax* malaria complications compared with patients without complications and 2) patients with chloroquine-resistant *P. vivax* compared with sensitive parasites from *in vivo* studies. To exclude patients with infection other than *Plasmodium*, all patients also had a blood culture performed for aerobic bacteria and serological tests for leptospirosis, HIV, viral hepatitis, and dengue. Quantitative real-time PCR was performed to compare the transcript levels of two main transporters likely to be involved in chloroquine resistance in *P. vivax*, namely the *P. vivax* chloroquine resistance transporter (pvcrt-o), and the *P. vivax* multidrug resistance transporter, (pvmdr-1). Twelve patients with *P. vivax*-attributed complications (severe anemia was the most common) and 10 mild symptoms were included. Furthermore, five resistant cases to chloroquine and five susceptible cases were enrolled. The complicated *vivax* group had a 8.5-fold increase in pvcrt-o levels expression as compared to the

control group with mild disease (p=0.009) and for pvmdr-1 no statistical difference was observed (p=0.566). Patients with chloroquine resistance had a 13.3 and 8.05-fold increase in pvcrt-o (p=0.005) and in pvmdr-1 (p=0.095) levels expression, respectively, compared to the susceptible group. These findings suggest that clinical complications as well as chloroquine resistant cases, due to *P. vivax* malaria could be associated with increased expression levels of the pvcrt-o gene likely involved in chloroquine resistance.

20. *Immune-Related BAT1, TNFA, and Il6 Gene Polymorphisms and* Plasmodium vivax *Malaria Outcome*

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BAT1 encodes a RNA helicase and is localized on chromosome 6 close to TNFA gene. BAT1 seems to down-regulate the expression of TNF-α and IL-6. In this study, we explore an association of SNP in BAT1, TNFA and IL6 and plasma levels of TNF-α, IL-6 and CXCL10 with *Plasmodium vivax* malaria. We studied 257 subjects, including 76 with symptomatic malaria, 104 with asymptomatic malaria and 77 non-infected individuals from the Brazilian Amazon. BAT1-22 C>G, BAT1-348 C>T, TNFA-308 G>A and IL6-176 G>C were analyzed by PCR and RFLP. TNF-α, IL-6 and CXCL10 plasma levels were measured by CBA. TNF-α, IL-6 and CXCL10 were elevated in symptomatic subjects (all p<0.0001) and no association was found between a single SNP and malaria (infection or disease). However, combination of genotypes GC/CC/GG/GG and GG/CT/GG/GG (BAT-22/BAT-348/TNF-308/IL6-176) are associated with lower and higher risk respectively, to develop symptomatic malaria when compared with asymptomatic and non-infected subjects (p=0.0428, OR:0.4145, 95% CI: 0.1746-0.9841 and p=0.0361, OR: 4.766, 95% CI:1.103-20.59 respectively). Heterozygotes for TNFA-308 and BAT-348 had higher TNF-α levels than wild type subjects (p=0.0347 and p=0.0215 respectively). CXCL10 levels were reduced in BAT-22 heterozygotes (p=0.0294) and GCC haplotype (TNF-308/BAT-22/BAT-348) causes a decrease in this chemokine (p=0.0071). Furthermore, AGC, ACC, GGT, AGT and ACT haplotypes were associated with a higher production of TNF-α (p=0.0248, p=0.0096, p=0.0266, p=0.0173 and p=0.0191). This study is the first to describe BAT1 and IL-6 SNP in *P. vivax* malaria. Our findings reinforce that a set of mutations in immune-related genes may influence inflammatory mediators and malaria outcome.

21. *Spectrum and Morbidity Pattern of Severe* Plasmodium vivax *Malaria and its Comparison with Severe* Plasmodium falciparum *Malaria (A Prospective Observational Study on Admitted Patients in PBM Hospital, Bikaner, India during 2010–2011)*

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Severe *Plasmodium vivax* malaria had been reported from Bikaner, India both in adults and children in PCR diagnosed *P. vivax* and *P. falciparum* monoinfection since 2005. Since then many retrospective and prospective studies from India, Brazil, Papua New Guinea, and Indonesia had reported similar cases. However very few studies had compared the clinical spectrum and morbidity pattern in comparison to *P. falciparum* malaria. In this study on 294 patients of malaria (*P. falciparum*: 175 and *P. vivax*: 119) diagnosed by PBF, RDT, and confirmed by PCR, the severe manifestation as defined by WHO was present in 136 patients (*P. falciparum*: 86 and *P. vivax*: 50). Mixed infections were not included in study because of very small number. All other co-morbid conditions were ruled out by

stringent clinical, biochemical and radiological examination. The severe manifestation of *P. falciparum* and *P. vivax* were jaundice 43 (50%) and 30 (60%), severe anemia 22 (25.58%) and 29 (58%), cerebral dysfunction 10 (11.63%) and 4 (8%), acute renal failure 11 (12.79%) and 9 (18%), thrombocytopenia 30 (34.88%) and 23 (46%) patients respectively. ARDS was observed only in one case of *P. vivax*. Multi organ dysfunction (MODS) was observed in 65 (75.58%) and 36 (72%) in *P. falciparum* and *P. vivax* respectively. The differences are statistically not significant except anemia in *P. vivax* malaria ($p<0.003$). Three patients died (*P. falciparum*: 2 and *P. vivax*: 1) having MODS. This study reaffirms the evidence of severe *P. vivax* malaria in this region having almost similar clinical presentation and morbidity pattern as seen in severe *P. falciparum* malaria.

22. *High Levels of Pyrethroid Resistance in Anopheles Funestus Population in Gembe Location, Suba District, Nyanza Province, Kenya*

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Background: Anopheles funestus is more efficient in contracting and transmitting malaria. Although it is difficult to rear, it is important to analyze field populations for pyrethroid insecticide resistance in Kenya. In Suba district Kenya, Malaria is endemic and prevalence is as high as 40%. In this study, we carried out small-scale field collections and rearing of An.funestus in Gembe location. The aim was to determine the pyrethroid insecticide resistance of wild caught populations in Suba District. Methology & principal findings: 910 indoor-resting mosquitoes were collected in five villages. 445 were An.funestus. The WHO susceptibility assays were unsuccessful due to difficulty in rearing the F_1 adults. However, sequencing for Kdr gene-TTA (L1014S) expressed high levels (60–90%) of mutations; indicating remarkably high pyrethroid insecticide resistance in An.funestus population in Gembe location. The GPS distribution pattern for Kdr gene frequencies observed, were suggestive of selection pressure to pyrethroid insecticides (Deltamethrin) widely used in LLINs and IRS. *Conclusion/significance:* The high levels of pyrethroid resistance observed in Gembe location, Mbita division are not only alarming, but a big challenge for resistance management strategies such as insecticide rotation in Gembe location, western Kenya. The selection pressure to pyrethroid insecticides complements the high malaria prevalence observed in this area.

23. *A 2012 Cross-Sectional Study of Malaria Infection and Gametocyte Prevalence in Thailand*

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A cross-sectional survey was performed in low/mid malaria transmission areas of Thailand near the Thai-Myanmar border. DNA and RNA were purified from finger-pricked blood samples taken from 4,358 local residents at the end of the 2012 malaria season. Sensitive genus-specific qPCR of blood DNA indicates an overall 5.5% infection rate. Most infections were asymptomatic with parasite density lower than 0.001% parasitemia. Geographic mapping shows a non-homogeneous distribution of infections. Age, sex, and bed net use data collected during the survey are being analyzed to identify potential association with infection. Parasite species identification by qPCR is on-going, as is gametocyte detection by qRT-PCR of sexual-stage Pfs25/Pvs25 transcripts. Results will be presented.

The study provides the most up-to-date report on local malaria infection in Thailand. Gametocyte prevalence data reflect the reservoir of transmission and are important for implementation of disease control and elimination programs.

24. *Comparison between Light Microscopy and PCR Method in Diagnosing* Plasmodium vivax *in Sudan*

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Although polymerase chain reaction (PCR) is a new technique in the diagnosis of malaria with very high accuracy; light microscopy is still conventional diagnostic method used in Sudan. In this study we compare the accuracy of light microscopy with the results of PCR as a gold standard. The blood samples were collected from 75 febrile cases infected with *Plasmodium vivax* in Eastern and Central Sudan, diagnosed by light microscopy and the slide were checked by expert microscopists. DNA samples were processed by PCR to amplify species-specific sequences of 18ss subunit ribosomal ribonucleic acid (18ssrRNA) genes of *P. vivax* and *P. falciparum*. The results showed that the positive slides for *P. vivax* based on microscopy were 66/75 (88%) *P. vivax* mono-infection, 3/75 (4%) were mixed infection of *P. vivax* and *P. falciparum*, 4/75 (5.3%) were not given DNA and 2/75 (3.7%) were *P. falciparum*. These findings concluded that Nested PCR is a useful technique in the detection of mixed malaria infection rather than the microscopy, and proved that the malaria microscopy required continuous training.

25. *Polymorphisms of Duffy Binding Protein of* Plasmodium vivax *from Timika, Papua, Indonesia*

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Plasmodium vivax Duffy Binding Protein (PvDBP), is a potential vaccine candidate targeting parasite invasion, however its genetic diversity may undermine the design of an effective vaccine. To investigate PvDBP genetic diversity, 83 *P. vivax* clinical isolates were collected from a hospital in Timika, Papua, Indonesia. *Pvcs* and *Pvmsp3*α genes were genotyped to identify unique polymorphisms. Nineteen isolates were selected based on the restriction fragment length polymorphism (RFLP) patterns of the *Pvmsp3*α and further tested for PvDBP polymorphisms by DNA cloning and sequencing. Sixty sequences of PvDBP region II derived from 19 selected isolates were analyzed and compared with other 527 PvDBP sequences worldwide. Sequence analyses of nucleotide and amino acid of PvDBPII demonstrated that *P. vivax* populations were genetically highly diverse, as shown by the large number of substitutions found in the DBP region II compared with the reference strain, Sal-1. Mutations were dominated by non-synonymous substitutions resulting in amino acid changes in the critical region of this domain. Analysis using dNS/dS ratio indicated that PvDBP is a subject of positive natural selection. The phylogenetic-tree analysis showed that PvDBPII of Timikan isolates clustered together with isolates from other geographic regions, including Thailand, Brazil, Sri Lanka, Papua New Guinea (PNG), Iran, and India.

Population differentiation analysis of PvDBPII worldwide revealed that PvDBPII of Timikan and Thailand isolates had lower *Fst* value (0.02768; $P<0.05$) than PNG isolates (0.05496; $P<0.05$). The results suggested lack of gene-flow between Timika and PNG compared to Thailand isolates. Analysis of the 10 most prevalent non-synonymous SNP that altered amino acids positions at 308, 371, 384, 385, 386, 390, 417, 424, 437, and 503 in PvDBPII using STRUCTURE program revealed five distinct

sub-populations among all 587 PvDBPII tested. The clustering of PvDBPII sequences showed the conservation of this protein between different countries. Further studies are is required to determine the importance of different PvDBP haplotypes in inducing immune response to this antigen. In view of the high level diversity of PvDBP, the identification of conserved motifs in this protein linked with its functional property is essential if a PvDBP-based vaccine is going to be developed.

26. *Genetic Diversity of* Plasmodium vivax *in Two Indonesian Islands*

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Information on the genetic diversity and structure of malaria parasite populations offers important insights into parasite transmission dynamics that can facilitate optimization of strategic malaria control activities. This information is especially useful for *Plasmodium vivax* which has proven more difficult to eliminate than *Plasmodium falciparum* owing in part to the challenge of relapsing infections. However, information on *Plasmodium* diversity is lacking for most of Indonesia. To set the foundations for further studies of *Plasmodium* diversity in the Indonesian archipelago, we characterized the genetic diversity and structure of *Plasmodium vivax* in two different malaria-endemic regions, Bangka and Sumba islands. Genotyping was undertaken using a consensus approach agreed with partners in the Asia Pacific Malaria Elimination Network (APMEN). Briefly, five microsatellite markers were genotyped in *P. vivax* clinical isolates collected from Bangka (66 isolates) and Sumba islands (26 isolates) using capillary electrophoresis with internal LIZ600 size standards.

Expected heterozygosity (He) was high in Bangka (He = 0.803) and in Sumba (He = 0.833), indicating highpopulation diversity in both regions. Within-host diversity, as determined by the Multiplicity of Infection (MOI), was higher in Sumba (median MOI: 1.88, range 1–3) than Bangka (median MOI: 1.44, range 1–3), consistent with higher transmission in Sumba. Despite differing transmission intensity, the degree of genetic differentiation between the two islands was very low (*Fst* =0.0256). These findings may reflect frequent migration between the two islands, potentially explaining the maintenance of high population diversity in Bangka despite apparent low transmission. Alternatively, the markers used may not have been sufficient to detect population structure in this endemic setting. Extensive gene flow may present an important challenge to *P. vivax* elimination efforts in the Indonesian archipelago. Further studies are required to investigate the population genetic diversity and structure of *P. vivax* in Bangka, Sumba and other endemic areas in Indonesia using dense SNP markers.

27. *Control Measures and Malaria Persistence in Mangalore City in Southwestern India*

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Although there were no reports of malaria prior to 1990 in Mangalore (12° 52'N 74° 53'E), thereafter the city has become a major hub for malaria in Southwestern India, coinciding with a rapid burst of construction activities. Since then malaria has steadily increased in parallel with increasing construction works and construction workers coming from malaria-endemic North-eastern regions. Mangalore has a population of ~0.5 million and malaria is holoendemic with *Plasmodium vivax* predominating and the annual parasitic index is ~11.8. Malaria is highest among construction workers (~25%), hotel workers (~18%) and hostel inmates (~9%), who together represent ~7% population of the city. Alarmed with an unusually high malaria infections and fatalities in 1995, a Malaria Control Action Committee was established by a group of physicians in alliance with government and private

organizations. Subsequently, in 2003, Mangalore City Corporation started a Malaria Cell to combat malaria. The control measures implemented were mainly focused on high-risk groups through radical treatment to fever, active sampling, distribution of insecticide bed nets, and identify and follow up migrant population. The control measures also include passive and active surveillance, strengthening Information Education Communication activities, anti-larval operations, and fogging at regular intervals. Despite these targeted interventions, malaria has progressively raised until 2008 (~10,000 cases/year) with a slight decline after 2008, which appears to be related to under reporting by private hospitals and labs. Therefore, there is a need to have further well planned and effective control strategies, and creating awareness among people at large to eradicate/effectively control malaria.

28. *Proteomics of* Plasmodium vivax *Salivary Gland Sporozoites*

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Malaria infections of mammals are initiated by the transmission of *Plasmodium* salivary gland sporozoites from an Anopheles mosquito vector. Comprehensively elucidating the protein composition of sporozoites will be invaluable in identifying targets for blocking malaria transmission. Previous efforts at identifying the proteins present in *Plasmodium* mosquito stages have been hampered by the technical difficulty of separating the parasite from its vector; without effective purifications, the large majority of proteins identified were of vector origin. Recently, we successfully combined improved sporozoite purification and high mass accuracy mass spectrometry to facilitate the most complete proteome coverage to date for purified salivary gland sporozoites from two *Plasmodium* species: human-infective *P. falciparum* and rodent-infective *P. yoelii.* Here the technique was applied to human-infective *P. vivax*. We detected 1,680 proteins with >85% identified with multiple unique peptide spectra. Proteins were analyzed in comparison to the previously reported *P. falciparum* and *P. yoelii* sporozoite proteomes. Two hundred and fifteen (13%) out of 1,680 proteins were expressed only in *P. vivax* sporozoites and among which 41% were unique to *P. vivax*, as defined by the absence of *P. falciparum* orthologs. These proteins may play some unique role in the biology of *P. vivax*. In summary, our results present the first and most comprehensive, high quality profile of proteins expressed in *P. vivax* salivary gland sporozoites. This new and valuable data may help in the identification of new candidates for anti-malarial drugs and vaccines.

29. *Immune Response of Anopheles Aquasalis to* Plasmodium vivax: *A Study of the Biology of an Amazon Parasite-Vector Interaction*

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Anopheles aquasalis is the most important vector of malaria in the South America coastal regions being in Brazil, an important transmitter of *Plasmodium vivax*. This study aims examining molecules that participate in the immune response during interaction between these two organisms. Subtractive libraries show expression of serine protease, fibrinogen, carboxypeptidase and bacteria responsive protein genes that were confirmed using real time PCR. The expression of a cecropin decreased after infection (a.i) while a serpin presented increased expression. Analysis of GATA revealed an increase in RNA levels 36h a.i. Silencing of this gene produced increased infection. In parallel, A. aquasalis cDNA sequences for the JAK-STAT pathway [transcription factor STAT, protein inhibitor of activated STAT (PIAS) and nitric oxide synthase (NOS)] and for genes related to cellular detoxification (catalase

and two superoxide dismutases) were obtained using degenerate primers. Also, STAT, PIAS and NOS are induced by infection and reverse genetics experiments have shown that this pathway is important in the immune response of A. aquasalis against *P. vivax*. Increase in mRNA expression of detoxification enzymes was observed 36h a.i. and decrease in activity 24h a.i. This increase may be related to the attempt of cells to decrease the amount of intracellular ROS due to the diminished activity of these enzymes 24h a.i. Surprisingly; the silencing of catalase exacerbated the infection. In conclusion, we showed for the first time in a New World vector-parasite pair, immune mechanisms adopted by A. aquasalis to combat *P. vivax*, providing information about molecules involved in the process of interaction and immunity.

30. *Molecular Characterization of the* Plasmodium vivax *vir Multigene Family in the Indian Populations*

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Plasmodium vivax vir gene family encoding variant surface proteins is the largest paralogous multigene family located in the subtelomeric region of different chromosomes. The functional role of this gene family is unknown, however variant surface proteins are immunovariant in natural infections that indicates for their putative role in establishing a chronic infection through antigenic variation. Unraveling sequence variations in the members of the vir gene family would provide valuable information to understand the nature and extent of genetic diversity within and between geographical regions. We have characterized vir gene family for 30 Indian *P. vivax* field isolates from Delhi, Nadiad (Gujarat), Panna (Madhya Pradesh), Rourkela (Odessa), Kamrup (Assam) and Chennai (Tamil Nadu). Vir-D and vir-G sub families were PCR amplified, cloned and 48 positive clones per samples were sequenced with 2x coverage. Maximum-Likelihood phylogenetic analysis shows huge genetic repertoire of vir-D and vir-G sub-families in *P. vivax* isolates regardless of their geographical origin. Among the analyzed samples, a range of 39 to 48 and 26 to 30 different sequences were observed per isolate for vir-D and vir-G respectively. Phylogenetic tree derived from vir-D sequences indicates that isolates from different geographical regions of India shared their genetic pool up to certain extent. This study provides useful information about the complex population structure of *P. vivax* on the basis of vir multigene family.

31. *Role of Cytokine-Mediated Endothelial Activation Pathway in Pathogenesis of Complicated* Plasmodium vivax *Clinical Isolates from Pakistan*

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Plasmodium vivax is the prevalent malaria species contributing 70% of malaria burden in Pakistan. Though considered benign, complicated cases of *P. vivax* are consistently being documented from this region. It has been hypothesized that *P. vivax* utilizes cytokine-mediated endothelial activation pathway as a mechanism to manifest severe disease symptoms. Therefore, we aimed to test this hypothesis by designing a case control study using well-characterized groups of uncomplicated (n=100), complicated cases (n=82) and healthy controls (n=100). Concentrations of cytokines, TNF-α,IL-6, IL-10 and endothelial activation markers ICAM-1 (Intracellular adhesion molecule-1),VCAM-1(Vascular adhesion molecule-1) and E-selectin were determined by Enzyme-Linked immunosorbant assay (ELISA).Correlation of cytokines and endothelial activation markers was done using Pearson two way correlation matrix. Furthermore, the significance of these biomarkers as indicators of disease severity was also analyzed. The results showed that TNF-α, IL-10, ICAM-1and VCAM-1were 3-fold, 3.7 fold and 2 fold increased between uncomplicated and complicated cases while IL-6 and E-selectin was 1.8 and 1.2 fold decreased between the two groups. Comparison of healthy controls with uncomplicated cases showed showed no significant difference in TNF-α concentrations while IL-6,IL-10,ICAM-1,VCAM-1and E-selectin were found to be 3.5-fold,20-fold,3-fold,4-fold and10-fold elevated respectively. Furthermore, significant positive correlation was observed between TNF-α and IL-10, TNF-α and ICAM-1, ICAM-1 and VCAM-1.A Receiver operating curve (ROC) was generated which showed that TNF-α, IL-10, ICAM-1 and VCAM-1 were the best individual predictors of complicated *P. vivax* malaria. Therefore, it is concluded that cytokine-mediated endothelial activation pathway is the possible mechanism of pathogenesis in *P. vivax* and cytokine and endothelial activation markers may serve plausible biomarkers of complicated *P. vivax* infection.

32. *Prevalence of Drug Resistance Associated Mutations in Clinical Isolates of* Plasmodium vivax *from Southern Pakistan*

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Antifolate antimalarial drugs sulphadoxine-pyrimethamine (SP) are the mainstay of malaria control in Pakistan. Though, not recommended, it is still being used largely in the public sector against *Plasmodium vivax*. Extensive use of SP indicates possibility of accumulation of drug resistance associated mutations in SP binding sites of *P. vivax*, encoded by dihydrofolate reductase (dhfr) and dihydroptereoate synthethase (dhps) genes. The aim of this study was to identify baseline frequencies of single nucleotide polymorphisms (SNPs) and prevalence of SP resistance associated mutations in clinical isolates of *P. vivax* (n=131) from Karachi, Sindh and Balochistan province. Nested PCR followed by direct sequencing and comparison with wild type reference sequences was performed. In dhfr, mutations were observed at codons F57L, S58R and S117N/T while novel non-synonymous mutations were observed at codon positions N50I, G114R and E119K .Two mutations, N50I and 117T were observed for the first time in Pakistan. The 50I mutation signifies intra species recombination of *P. falciparum* and *P. vivax* while 117T, isolated globally from treatment failure cases, shows the extent of SP pressure on *P. vivax*. In dhps, mutations were observed at codon position A383G and A553G while non-synonymous mutations were observed at codon positions S373T, E380K, P384L, N389T, V392D, T393P, D459A, M601I, A651D and A661V. Results from this study provide evidence that increasing number of SP resistance associated mutant alleles, comparable to those reported worldwide, are circulating in southern Pakistan. These alleles may play a significant role in transmission of resistant strains via human parasite reservoirs exacerbating extensive drug pressure on *P. vivax* and possibly making SP defunct for future use.

33. *Experimental* Plasmodium vivax *Infection of Anopheline Species of Brazilian Amazon*

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Anopheles darlingi and Anopheles albitarsis are proven malaria vectors in the Amazon region. Anopheles nuneztovari and Anopheles triannulatus were found naturally infected with *Plasmodium vivax*, but their status as vectors is not well defined. In this study, these species were analised for their susceptibility to *P. vivax*. Larvae of the four species were field collected and lab reared until adult stage. Females were fed by membrane feeding using blood from malarial patients (Ethical Committee 3726). The effect of plasmodial infections (proportion of gametocytes) in human and its effect on the mosquito infection (rate infection and number of oocysts) were evaluated. The infection rates of A. darlingi, A. albitarsis, A. nuneztovari and A. triannulatus were 18.3, 45.4., 23.5 and 8.9, respectively. Inactivation of the blood serum before the mosquito feeding increased infection rates in all the species, except in A. albitarsis. The mean numbers of oocysts per midgut diminished in all species, but not in A. triannulatus. There was not correlations between the proportion of gametocytes in blood sample with infection rates and number of oocysts developed (Kendall`s Tau, R < 0,3). Anopheles albitarsis had higher infection rate than A. darlingi, but it was field collected in low numbers such as A. nuneztovari. Anopheles triannulatus was collected in high numbers, but its infection rate is very low. In conclusion, all the studied species were susceptible to *P. vivax* infections, but, probably, their contribution for malaria transmission in the Amazon region may be associated to their densities over the year.

34. *Contribution of* Plasmodium vivax *Hypnozoites to the Burden of Malaria Infection and Disease in Papua New Guinea Children*

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In Papua New Guinea (PNG), *Plasmodium falciparum* and *P. vivax* are co-endemic, with studies in East Sepik Province demonstrating a higher prevalence of *P. vivax* than *P. falciparum* in young children. Routinely administered anti-malarials, chloroquine and artemisinin combination therapies, act against the blood-stage of the parasite and do not eliminate *P. vivax* hypnozoites in the liver, resulting in a high rate of relapse. Currently, primaquine is the only licensed drug effective against liver-stage parasites, however due to several limiting factors, this drug is not in widespread use in PNG. To investigate the contribution of long-lasting liver-stage hypnozoites to the burden of malarial infection and disease, a treatment to re-infection study was conducted in children aged 5-10 years in the Maprik District of East Sepik Province. Briefly, 508 children were randomised to receive chloroquine (3d)/artemether-lumefantrine (3d) and primaquine (20d; 0.5mg/kg); or chloroquine (3d)/artemether-lumefantrine (3d) and placebo (20d). The children were actively monitored for re-infection every fortnight for 8 months and passive surveillance measures were also implemented. Preliminary analysis has revealed that 271 children (53.3%) became re-infected with *P. vivax* during follow-up. Of these, only 84 occurred in the 250 children that received primaquine (33.6% with recurrent parasitaemia) compared to 187 in those 258 that did not (72.5%, $p < 0.001$). The majority of these recurrent infections (69%) occurred within 12 weeks of treatment suggesting rapid activation of hypnozoites. Failure to adequately treat *P. vivax* infections with effective hypnocidal drugs will severely impede attempts to control *P. vivax* in PNG.

35. ***Immunogenicity of Recombinant Proteins and Adenoviruses Based on the Different Allelic Forms of the Circumsporozoite Antigen of*** **Plasmodium vivax** ***Aiming at the Development of a Universal Vaccine against Malaria***

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Introduction: Plasmodium vivax is the second most prevalent and most widespread species causing an estimated 132-391 million cases annually. The relative inefficiency of the measures currently used for control demands the development of new strategies for prevention such as vaccines. In the past 15 years, studies aimed at the development of a recombinant vaccine against the human malaria caused by the deadly parasite *Plasmodium falciparum* were based on the circumsporozoite (CS) antigen. In a recent publication, phase III trials in African children reported 50% efficacy of the recombinant vaccine.

Results and Conclusions: Based on the studies with *P. falciparum*, our studies aimed at the generation of bacterial recombinant proteins and replication defective adenoviruses expressing primary sequences from three different allelic variants of *P. vivax* CS protein. These recombinant proteins in combination with the adjuvant Poly(I:C) and adenoviruses were successfully used in protocols of homologous (recombinant protein/recombinant protein) or heterologous (adenovirus/recombinant protein) vaccination strategies in an experimental mouse model. Most importantly, these different vaccine strategies elicited antibodies which strongly reacted with the immunodominant regions of all three allelic variants of *P. vivax* CS protein. These recombinants proteins/adenoviruses and the respective protocol of vaccination were object of a patent application to the development of a universal vaccine against *P. vivax* malaria (USPTO IFW61614439). Supported by FAPESP, INCTV (CNPq) and CAPES.

36. ***Antibody-Based Flow Cytometry for Detection of Parasite in*** **Plasmodium vivax** ***Research***

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During the past decades, research on *Plasmodium vivax* has been focused on an establishment of in vitro continuous culture, new anti-malarial drug discovery and vaccine development. Detection of the parasitemia in the culture is a key measurement in the evaluation strategy for those experiments. Two detection methods have been widely used, counting the parasitemia under light microscope (standard method) and nuclear staining-based flow cytometry. Using light microscope is labor-intensive, time consuming, need microscopist who has been well trained and in-accurated due to small number of counted cells. Nuclear staining-based flow cytometry has been investigted to replace microscope. The fact that *P. vivax* preferrentially infect reticulocytes and a very low parasitemia complicate the detection and quantification by nuclear staining-based flow cytometry. A simple antibody-based flowcytometry method was developed for rapid parasite detection. The determination of parasitemia by antibody-based method correlated (P) with counts from Giemsa-stained thick blood smears (Spearman correlation coefficient = 0.94, $P \leq 0.001$). The antibody-based flowcytometry is a simple, robust and efficient method for detecting *P. vivax*-infected reticulocytes.

37. Plasmodium vivax *Sporozoite Challenge Studies in Colombia*

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Important efforts have been performed towards the development of malaria vaccines including *Plasmodium vivax* as well as to develop antimalarial drugs targeting parasite liver stages. Therefore, we have been working on the establishment of a safe, reliable, and reproducible sporozoite infectious challenge method in Colombia. Two infection trials have been conducted in naïve human volunteers that have been exposed to the bite of Anopheles albimanus mosquitoes infected with *P. vivax*. In the first challenge, volunteers where exposed to the bites of 2-4, 5-7 and 8-10 mosquitoes infected from a single parasite donor. In the second challenge, volunteers where exposed to the bites of 2-4 mosquitoes infected from different donors. We found that individuals from the first challenge developed pre-patent periods within a range of 9 – 13 days (mean 10.6), as determined by Thick Blood Smear (TBS), similarly in the second challenge pre-patent period ranged between 9-16 days (mean 12). All volunteers were closely followed for clinical appearance of malaria and where treated immediately parasitemia became patent. All participants successfully recovered from malaria after treatment, with no serious adverse events. We are currently conducting a third trial to determine differences between naïve and pre-immune volunteers, who will be exposed to 2-4 mosquito bites. This latter trial would be useful to determine differences between controls and immunized volunteers who develop partial protection. In conclusion, human naïve volunteers can be safely and reprodubly infected with as low as 2 mosquito bites. This method would accelerate the assessment of anti *P. vivax* drugs and vaccine candidates.

38. *Folate Pathway Possibly Associated with* Plasmodium vivax *Relapse in the Peruvian Amazon BASIN After Primaquine Treatment*

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Risk factors for *Plasmodium vivax* relapses after Primaquine (PQ) treatment have not been clearly identified yet. We studied the association between relapse and host factors and three *P. vivax* genes which would participate in possible PQ mechanisms of action: oxidative stress (Pvmdr1) and pyrimidine synthesis inhibition (Pvdhfr and Pvdhps). A case-control study was conducted within an Efficacy clinical trial of 3 different PQ doses to prevent relapses in three communities in the Peruvian Amazon basin. 47 homologous relapse cases and 401 controls were analyzed for clinical-epidemiological factors, and 57 randomly selected controls were analyzed for parasite genetic factors. We found that subjects with weight>58 Kg (HR 2.71, p=0.012) and living two sites, Padrecocha and San Juan, had increased relapse risk (HR 3.37, p=0.033 and HR 2.89, p=0.047 respectively), after adjusting by treatment arm and age. Concerning the parasite factors, the A383G Pvdhps genotype and the triple mutant Pvdhfr genotype S58R/Y69(TAT>TAC)/S117N were associated with relapse (OR 2.84, p=0.036 and OR 2.79, p=0.023 respectively), after adjusting by treatment arm. The Pvdhfr genotype remained marginally significant after adjusting by community and weight, despite the small sample size. Pvmdr1 was not associated with relapses. These findings suggest that mechanisms in folate pathway of the parasite, important for de novo pyrimidine synthesis, could be involved in relapses after PQ treatment. More studies are needed to validate these results.

39. *Prevalence, Enzyme Kinetics, and Variant Genotypes of G6pd-Deficiency at Sumba in Eastern Indonesia*

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G6PD deficiency (G6PDd) is a common enzyme disorder that seriously impedes malaria elimination goals by rendering these patients highly vulnerable to serious harm caused by primaquine. At three districts in Sumba island in eastern Indonesia, 2031subjects were screened for G6PDd using the Trinity Biotech quantitative test and the deficient samples (105 subjects, 5.2%) were further analyzed biochemically for purified enzyme kinetics and genotyped as well. Kinetics data showed enzyme activity that deviated from normals and DNA analysis showed 3 common variants dominated the samples: Vanua Lava (14233 T>C), Viangchan (16381 G>A), and Chatham (16512 G>A). These 3 variants all fall in Class II according to the WHO enzyme classification which is considered as severe, i.e., very vulnerable to harm caused by primaquine. One new mutation was found at position 17089 T>G changing cysteine to glycine and the kinetic data indicated that this enzyme had a higher preferance for NADP than normal. There were also 3 SNPs found in the normals as well as in deficient samples, two of which were already registered at the SNP database (rs 2230037 and rs 1050757). These findings inform the weighing of risk and benefit with respect to primaquine policy and practice in striving to reach Indonesia's declared malaria elimination goals.

40. *Characterization of the Nuclear Encoded Hypothetical Proteins Targeted to the Apicoplast in* Plasmodium vivax

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Plasmodium vivax causes more than 70% of human malaria cases in Central and South America, South-East Asia and India. Apicoplast, a plastid like organelle, harbored by *Plasmodium* is being looked upon as a putative drug target. A number of nuclear encoded proteins tagged by an N-terminal bipartite leader sequence are believed to be transported to apicoplast. These proteins are believed to participate in various house-keeping processes, such as apicoplast genome replication, transcription or translation and in various metabolic pathways functional in this organelle. A number of such proteins still remains to be hypothetical. Sequences of such *P. vivax* nuclear encoded hypothetical proteins were analyzed for the presence of apicoplast targeted leader sequences using PlasmoAP and PATS. Based on the conserved functional domains (using Conserved Domain Detection, NCBI) two cellular proteins were shortlisted: PVX_111125 — Similar to 16S rRNA processing protein possessing a conserved Ribosome Maturation (RimM) domain; and PVX_001980 — Similar to Zinc regulated transporter/Iron regulated transporter like proteins. Both the genes were amplified, sequenced and cloned from *P. vivax* infected field samples. Sequence analysis showed major resemblance with putative RimM protein sequences and zinc transporter domain respectively from other apicomplexan and bacterial species. An "RNA binding motif" GXXG was found to be conserved in PVX_111125. Phylogenetic analysis showed close association with orthologues from primate parasite. The functional domains and structure of PVX_111125 protein were elucidated using various bioinformatic tools which suggests the role of protein in Ribosome maturation.

41. *Experimental Infection of Anopheles Aquasalis with* Plasmodium vivax: *A Useful Parasite-Vector Model for Studying Amazon Vectors*

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Malaria affects 300 million people worldwide causing 1.5 to 2.7 million deaths. In Brazil, approximately 450,000 cases are annually reported mainly of *Plasmodium vivax* malaria. Anopheles aquasalis is present in several South America countries and is the most important vector of malaria in the coastal regions of Brazil transmitting the etiological agent *Plasmodium vivax*. In order to have a well-reputable laboratory experimental model for analyzing several aspects of the biology of the interaction of *P. vivax* with New World mosquito vector, we established sequential morphological studies to observe the parasite cycle inside the vector. Firstly, we developed in vitro infections of A. aquasalis with *P. vivax* infected-blood from malarial patients. The blood samples were selected by the presence of circulating gametocytes, the parasite form that is able to start infection in the vector. At different period of times, until fifteen days after the infected blood meal, the entire infected mosquitoes or their dissected midguts were processed for observation by different morphological techniques. Conventional optical, scan and transmission microscopies, as well as, laser confocal microscopy allowed us to observe details of the parasite cycle inside the vector as the invasion of the midgut cells, oocyst formation outside the midgut, sporozoite liberation from oocysts and the invasion of the salivary gland. These observations showed the complete life cycle of the parasite after experimental infection of A. aquasalis with *P. vivax* and enforce this parasite-vector model as useful tool for studying Amazon vector.

42. Plasmodium vivax *Chloroquine Efficacy Studies Confirm Drug Susceptibility in Chennai, India*

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Plasmodium vivax is endemic throughout India, with more than 645,000 cases reported in 2011 by the National Vector Borne Disease Control Programme. Assessing the *P. vivax* burden in India is compounded by the continual threat of an emerging chloroquine resistant parasite population from neighboring countries in Southeast Asia. Chennai, the capital of Tamil Nadu represents an urban setting for *vivax* malaria in southern India, and was selected as the sentinel site for investigating chloroquine sensitivity in *vivax* infections. In this study, chloroquine sensitivity was measured by in vitro chloroquine drug assay (N = 68) and the therapeutic efficacy of chloroquine evaluated by an *in vivo* study (N =125), followed by evaluation at the molecular level of the *P. vivax* mdr1 gene and eight genome wide microsatellite loci. Chloroquine resistance was not detected in the parasite population using either assay; however, valuable population-level diversity information was obtained, revealing a high rate of relapsing infections.

43. *ARDS in* Plasmodium vivax *Malaria*

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Acute renal failure, disseminated intravascular coagulation (DIC), acute respiratory distress syndrome (ARDS), hypoglycemia, coma, or epileptic seizures are manifestations of severe *Plasmodium falciparum* malaria. On the other hand, *Plasmodium vivax* malaria seldom results in pulmonary damage and pulmonary complications are exceedingly rare. We report the case of a 52-year-old male living in a malaria-endemic area on the western coast of India, who presented with ARDS and was diagnosed as having *P. vivax* malaria. A diagnosis of *P. vivax* malaria was established by quantitative buffy coat (QBC) method which showed 1+ parasite load. It was also confirmed by parasite specific

lactate dehydrogenase (p LDH) by malaria card test (RTD), which is a screening antigen-antibody reaction test considered as 100% sensitive and 99.77% specific for *P. vivax*. *P. falciparum* malaria was ruled out as histidine related protein (HRP-2) was negative. His 2D echocardiography showed normal LV function; serum cortisol was within normal limits; with no evidence of renal failure or pancreatitis or sepsis or multi organ dysfunction, All other possible causes for ARDS were ruled out. Hence ARDS was considered as a complication of *P. vivax* malaria per se. After specific anti-plasmodial therapy and intensive supportive care with NIPPV, the patient gradually recovered and was discharged from the hospital.

44. *The Contribution of Animals in Malaria Transmission in Village Area, Nusa Tenggara Timur, Indonesia*

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Nusa Tenggara Timur is one of the provinces in Indonesia who has a high prevalence of malaria. The existence of animals around the house could contribute in transmission malaria. To understand the role of animals in malaria transmission, therefore, it is important to determine the risk of the existence of animals in village area of this province. A total of 38,000 households from the secondary data 'Basic Health Research' conducted by Ministry of Health 2007 analyzed used Chi Square Test and Logistic Regression to calculate the risk of any size of animal in malaria transmission. The independence variables were the size of animals which divided into four groups of animal, namely big animal (cow, horse, and buffalo), medium animal (pig, sheep, and goat), small animal (cat, dog, and rabbit) and poultry (chicken, bird, and duck) and the existence of cage, while the dependence variable was malaria itself. The middle animals, small animals and poultry had p value less than 0.05, whereas big animals had no significant p value ($p > 0.05$). The existence the cage in the household also showed a risk in transmission with OR= 4.71 (3.63–6.10, $p < 0.05$). The multivariate logistic regression analysis revealed that all of big animals, middle animals, small animals, and poultry were the risk factors of malaria with OR 0.80 (0.72–0.89), 1.32 (1.19–1.44), 1.12 (1.03–1.22), and 1.158 (1.05–1.28) respectively. The big animals contributed as a protective factor to malaria, but middle animals, small animals, poultry and having a cage was a risk factor.

45. *Congenital* Plasmodium Vivax *Malaria: An Observational Prospective Study from Low Endemic Region (Bikaner, India)*

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Congenital malaria is defined as malaria parasitaemia in the first week of life. Reports of congenital malaria in *Plasmodium vivax* are very scanty. In this context to describe the occurrence and clinical spectrum of congenital *vivax* malaria in Indian perspective, this prospective study was conducted on admitted neonate from January 2011 to December 2012. The species diagnosis was done by peripheral blood smear examination and rapid diagnostic test. Other investigations were done as clinically indicated. A total of 1168 new born admitted in first week of life were screened. Out of them 23 (1.97%) had evidence of parasitaemia (*P. vivax:* 17 and *P. falciparum:* 6). The criteria for admission in these 17 neonates with congenital *vivax* malaria were LBW and prematurity (41.18%), septicemia (35.29%), perinatal asphyxia (17.65%), jaundice (17.65%) and seizures (5.88%). The clinical malaria was seen in 14 (82.35%) neonates in which spectrum was anemia (76.47%), fever (64.70%), hepatosplenomegaly (58.82%), thrombocytopenia (58.82%) and poor feeding/ lethargy/ irritability (52.94%). Although the presence of parasitaemia didn't differ the proportion of neonates having fever (χ^2=0.338; p=0.56) and hypoglycemia (χ^2=0.117; p=0.732) from those without parasitaemia, but it was significantly associated with anemia (Hb <10 gm/dl) (χ^2=10.996; p=0.001).

The mean Hb level was 8.6±3.2 gm/dl; mean platelet count was 139025.32±86236.56/µl; mean reticulocyte count was 4.2±1.6%; and mean parasite density was 11855.38±4123.21/mm^3. All these neonates were treated according to WHO guidelines and none of them expired. Thus, this study emphasizes the occurrence of *P. vivax* congenital malaria even in low transmission area and without typical manifestations.

46. *Clinical Spectrum and Morbidity Pattern of Severe* Plasmodium vivax *Malaria in Children: An Indian Perspective*

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Recent observations from different parts of world have confirmed the existence of severe *Plasmodium vivax* malaria in children. This clinico-epidemiological study describes the occurrence and spectrum of severe *P. vivax* malaria in children in Indian perspective. This prospective study was conducted on 1269 admitted children of malaria from January 2010 to November 2012. The species diagnosis was done by peripheral blood smear and rapid diagnostic test. Polymerase chain reaction confirmation on 100% of severe *P. vivax* malaria revealed 98.55% accuracy. Severe malaria was defined strictly on WHO criteria (2000). The possibilities of other disease/infections causing similar illness were investigated thoroughly and stringently. In this cohort study, the proportion of P.*falciparum*, *P. vivax* and mixed malaria was 48.89%, 42.57% and 8.52% respectively. Severe malaria was present in 50.47% children, with the highest relative risk among *P. vivax* monoinfection (62.96%) compared to *P. falciparum* monoinfection (40%; RR=1.574 [95% CI 1.414-1.767], p<0.0001) and mixed infections (38.89%; RR=1.619 [95% CI 1.276–2.213]). Severe anemia (81.18%) was the major severe manifestation of severe *P. vivax* malaria followed by thrombocytopenia (70.59%), hepatic dysfuction (32.94%), cerebral malaria (16.47%), renal dysfunction (15.29%), abnormal bleeding (11.18%), and acute respiratory distress syndrome (ARDS) (7.65%). Multiorgan dusfunction was seen in 47.65% children. The proportion of all these severe manifestations were highly significantly in <5 years age children (p<0.001). The case fatality rate of severe *P. vivax* malaria was 2.9% in comparison to 3.5% of severe *P. falciparum* malaria (p=1.0). This study reaffirms the evidence of potential of *P. vivax* monoinfection to cause severe malaria in children in Indian perspective.

47. *Associated Factors to* Plasmodium vivax *Malaria Infections in the Peruvian Amazon*

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The aim of this study was to assess the factors that are associated to individuals with *Plasmodium vivax* infections in different locations of the Peruvian Amazon. Forty five patients, older than 16 years old, were enrolled during August to November 2008. Regards to demographic characteristic, it was found that 8 (17.8%) came from rural area (communities near to Napo and Chambira Rivers), 29 (64.4%) from a peri-urban area (San Juan and Belen) and 8 (17.8%) from an urban area (Iquitos and Punchana). The parasitemia levels reached numbers of 1,980.63 to 25,596.92 parasites/µl, from them only 5 (11.1%) patients showed higher parasitemia than 10,000 parasites/µl. 41 (97.7%) patients of this group presented classical malarial symptoms such as chills, fever and sweating; and the remaining individuals presented no symptoms related to malaria. Unfortunately, we do not know the number of previous episodes for each case in order to correlate this with the development of immune system. This study, also, found that more men than women presented the infection, possibly because they are the ones who travel or go out to work and are exposed to the vector, while women stay at home. Therefore, this study suggests that living in a peri-urban area predisposes individuals to infection with *P. vivax* in a higher proportion than *P. falciparum*, that is present in the peri-urban areas but in a lesser proportion.