

Focus on *Plasmodium vivax*

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In Bangkok, Thailand, 3–8 February 2002, the Multilateral Initiative on Malaria convened the first malaria conference, *Vivax Malaria Research: 2002 and Beyond*, devoted entirely to *Plasmodium vivax* research.

Plasmodium vivax accounts for over half of all malaria infections outside Africa and 10% of those in Africa (an estimated 75 million acute episodes per year). *Vivax* malaria is prevalent in many regions of Asia and the Latin American subcontinent, and there are a few places where *P. vivax* is transmitted exclusively. Participants at this recent meeting were gratified by the long overdue attention to *P. vivax* malaria. In recent years, a well justified increase in support for *Plasmodium falciparum* research has occurred, whereas *P. vivax* research has lagged disproportionately. The often offered assumption that information about vivax could be extrapolated from falciparum research was challenged by several presentations at this meeting.

Human evolution

Before the advent of antimalarial drugs, *P. vivax* malaria was a chronic, disabling and cachectic disease, widespread in temperate North America and Europe as well as in the tropics. This disease is severe enough to select for antimalarial human mutations. Data accumulated from death records in the 1800s in Essex, UK, demonstrated that *P. vivax* probably decreased the average life span from 58 to 33 years.

During the last ice age, *P. vivax* transmission in West Africa provided selective conditions for driving the Duffy red blood cell antigen (now known to bind a *P. vivax* merozoite surface protein) deficiency mutation to near fixation in the endemic population. A new Duffy antigen polymorphism that reduces parasite binding has recently been identified in Papua New Guinea, indicating that the selection process continues.

Plasmodium vivax morbidity

The role of 'bed shaking' paroxysm levels of inflammatory cytokines and tumour necrosis factor (TNF) α , which are often

higher than those found in severe *P. falciparum* malaria cases, and 'anti-disease' nitric oxide are being examined in acute *P. vivax* episodes. A transient lipid factor that induces leukocyte aggregation and cytokine production has been isolated from plasma taken during paroxysms. Many of the severe pathologies attributed to sequestration of *P. falciparum* during infection are also observed in *P. vivax*. Acute *P. vivax*-associated respiratory pathogenesis similar to that seen in *P. falciparum* was described, suggesting a common inflammatory mechanism. Despite low parasitemias, *P. vivax* also induces anemia, especially in young children. A study of >11 000 pregnancies in refugee camps on the Thai–Myanmar border demonstrated that *P. vivax* infection was associated with an increased risk of low-birth-weight babies. More research is needed to understand the mechanisms of *P. vivax* morbidity.

P. vivax–*P. falciparum* interactions

New observations, which add to the extensive literature on interactions between *P. vivax* and *P. falciparum* (Pv–Pf) infections, were discussed. In a study conducted in Papua New Guinea, *P. vivax* was the most common species infecting children under six months old, whereas *P. falciparum* prevalence was low in the first six months then increased to ~30% by the age of one year. Mixed infections in 34 asymptomatic children observed over 60 days showed non-independent sequential episodes of infection with each species, suggesting that cross-species, density-dependent regulation of parasite load is occurring. PCR studies from many parts of the world indicate that *P. vivax* is significantly underdiagnosed microscopically in mixed infections. In Thailand, *P. vivax* was present in 30% of acute *P. falciparum* cases, and covert *P. falciparum* was present in 8% of acute *P. vivax* cases, although only 1% mixed infections were reported by standard microscopy. In these mixed infections, the risk of severe malaria was reduced fourfold, gametocyte carriage was reduced 3.5-fold, the risk of anemia was reduced 1.8-fold and

recovery from anemia was more rapid. The implications for malaria control strategies of Pv–Pf interactions should be considered.

Vaccine development

Participants strongly endorsed promoting combined Pv–Pf vaccines as necessary for most malaria endemic regions (Box 1). However, there was no consensus on how and where *P. vivax* antigen or combined vaccines should be field tested. Human trials of a *P. vivax* circumsporozoite (CS) peptide vaccine, Duffy binding protein, MSP1-p19 and the transmission blocking antigens Pvs25 and Pvs28 as vaccine candidates are being planned. Numerous *P. vivax* red blood cell stage antigens have been characterized using primate models and *P. vivax*-related simian malaria parasites. Primate models of *P. vivax* could be more effectively used for studying vaccines, drugs and *P. vivax*-associated pathology. The lack of a long-term *in vitro* culture method for *P. vivax* remains the largest barrier to research progress. Improved techniques for short-term culture for drug sensitivity testing, culturing *P. vivax* liver stages in human hepatocytes and the production of ookinetes from infected blood were also reported.

Drugs against malaria

The identification of *P. vivax* chloroquine resistance as therapeutic failure is significant in parts of Indonesia and Guatemala. However, surprisingly little resistance is reported in areas with high levels of *P. falciparum* drug resistance such as Thailand. Resistance to chloroquine appears to be a result of a mechanism different from that in *P. falciparum*. In other comparative molecular studies, *P. vivax* resistance to anti-folate drugs appears to be a result of the same mutations in the gene encoding dihydrofolate reductase thymidylate synthetase (DHFR). Innate sulfadoxine resistance in *P. vivax* is associated with a sequence polymorphism at the drug-binding site of the target enzyme. The lack of effectiveness of the standard primaquine treatment in Australian peacekeepers returning from East Timor

was chronicled: tafenoquine, an experimental drug with liver-stage activity, was found to be no more effective than primaquine in these cases. Currently, the curative primaquine

regimens used are highly variable and there are several reports of resistance. The lack of effectiveness of five-day and 14-day lower dose regimens was substantiated, but a 14-day regimen of

30 mg per day was reported to be effective in most cases. It was suggested that total primaquine input is the key to effectiveness, and shorter regimens of higher daily doses that could inspire better compliance should be tested. A major barrier to the use of higher primaquine doses is the lack of a simple glucose 6-phosphate dehydrogenase (G6PD) deficiency assay to predict hemolytic toxicity.

Genetic diversity of *P. vivax*

The impact of the extensive genetic diversity observed in *P. vivax* is being examined. The two main mosquito vectors in Mexico have different susceptibilities to the two *P. vivax* CS phenotypes related to ookinete development and migration in the mosquito midgut, which explains their separate geographic transmission patterns. Extensive *P. vivax* genetic diversity was reported within individual patients, even in areas with low entomological inoculation rates (e.g. ~35% *P. vivax* infections in Thailand contained mixed genotypes). *Plasmodium vivax* sequences contain extensive synonymous polymorphisms, whereas non-synonymous mutations predominate in *P. falciparum* genes. The *P. vivax* variant antigen (*vir*) genes, a multigene family of 600–1000 copies, are being characterized. The highly conserved *P. vivax* merozoite antigen with erythrocyte binding locus (MAEBL) protein is expressed in the rhoptry and on the surface of mature liver stages, merozoites and sporozoites, with soluble and transmembrane forms generated by alternative RNA splicing. Preliminary reports on the long awaited *P. vivax* genome sequencing project offer great promise for enhanced research on this neglected parasite.

Future directions

Delegates met in small groups on the final day to draft recommendations to specifically enhance *P. vivax* research in the areas of pathology and morbidity; immunity, antigens and vaccines; drug treatment, resistance and development; epidemiology and transmission; and *P. vivax* diversity and genome studies (Box 1).

Recommendations, abstracts and presentations from the meeting can be found on the MIM website (http://mim.nih.gov/english/events/vivax_research_conf/index.htm). A series

Box 1. Recommendations for enhancing *Plasmodium vivax* research

Research needs of fundamental importance

Inexpensive, rapid and accurate *Plasmodium vivax*-specific diagnostic tools for detecting low parasitemia in mixed malaria infections.
Basic research and intervention assessments require long-term *in vitro* cultures and cryopreservation methods are needed for all *P. vivax* life cycle stages.
Accelerate basic biological research and development of drugs and vaccines through increased use of primate models for vivax malaria and closely related simian malaria species such as *Plasmodium cynomolgi*.
Availability of *P. vivax*-specific standardized reagents (e.g. molecular markers, recombinant antigens and monoclonal antibodies).
Address the paucity of basic knowledge on the unique biological characteristics of *P. vivax*, such as relapse, reticulocyte multiplication and early gametocytemia.
Increased collaboration between endemic and non-endemic areas, and the generation of *P. vivax* researchers is crucial for sustained and productive *P. vivax* research.
Unique treatment and prevention strategies to control *P. vivax* malaria.

Pathology and morbidity research

Clinical epidemiological studies in different transmission settings to measure spectrum and magnitude of *P. vivax* disease manifestations and immunity, with specific attention to the contributions of relapsing infections and emerging drug resistance.
Molecular mechanisms involved in *P. vivax*-associated pathology and morbidity, especially those related to fever, anemia and pregnancy.
Examine interactions during *P. vivax* and *P. falciparum* co-infections (i.e. cross-protection and pathophysiology).
Defining the socioeconomic burden of *P. vivax* acute and chronic disease.

Immunity, antigen and vaccine research

Establish the global goal of a combined *P. vivax*–*P. falciparum* vaccine because *P. vivax* transmission co-exists with *P. falciparum* transmission.
Discovery of *P. vivax* vaccine candidate antigens and a development pipeline (not necessarily dependent on *P. falciparum* vaccine research).
Expand studies of natural *P. vivax* diversity, immunity and markers of protection in endemic populations using standardized reagents.
Extensive discussion and analysis of how and where to test single *P. vivax* vaccines and/or in combination with *P. falciparum* vaccines.

Drug treatment, resistance and development research

Improve and standardize methods to assess drug susceptibility in culture and in patients.
Determine the molecular mechanisms of resistance for conclusive epidemiological studies of resistance.
Studies to determine the optimum dose and duration of primaquine in different endemic regions.
Simple, rapid and inexpensive assays to identify individuals likely to suffer hemolysis to inform primaquine treatment decisions.
Increase in drug discovery research for liver-stage parasites.

Epidemiology and transmission research

Epidemiological understanding of *P. vivax* transmission will require analysis of personal infection risk factors, and the mapping of risk factors on a small and a large geographical scale over time.
Studies of the impact of human movement on *P. vivax* transmission.
Systematic gathering of epidemiological data on *P. vivax* and made widely available in standardized, shared databases.
Research on *P. vivax* interactions with vector mosquitoes and dynamics of transmission.

Genetic diversity and genomic studies

Comparative genomic studies for identifying unique *P. vivax* genes involved in pathogenicity and as new therapeutic targets.
Identification of several reagents of paramount importance for genomic and genetic diversity studies (e.g. *P. vivax* and *P. cynomolgi* genomic DNA, stage-specific complementary DNA libraries, pulse-field gel chromosome blots and genomic DNA libraries of major mosquito vectors).
Training and training resources in bioinformatics and epidemiological analysis methods in endemic countries.
Good quality DNA sequence data and supportive documentation deposited in public databases because of the lack of available parasite material.
Forming a network or consortium of researchers interested in *P. vivax* diversity and population genetics studies.

of peer-reviewed articles expanding on various aspects of *P. vivax* research presented at this meeting will be published in *Trends in Parasitology*.

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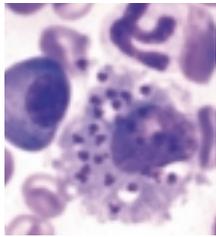
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Canine leishmaniasis: novel strategies for control

Richard Reithinger and Clive R. Davies

The 2nd International Forum on Canine Leishmaniasis was held in Seville, Spain, 6–9 February 2002.



The leishmaniases are a group of (mainly) zoonotic diseases transmitted to humans and animals by the bite of phlebotomine

sandflies. Currently, in terms of global disease burden, the leishmaniases are the third most important vector-borne disease (after malaria and lymphatic filariasis), responsible for an estimated 1.81 million disability adjusted life-years (DALYs) and 57 000 deaths annually. The latest findings of canine leishmaniasis diagnostics, epidemiology and control were presented and the implications for human disease were discussed at this meeting.

Changes in transmission epizootology

Over the past 20–30 years, there has been a dramatic increase in the number of reported human leishmaniasis cases, a trend that shows no signs of abating. This trend is partly due to better diagnosis and notification of leishmaniasis cases, but there is increasing evidence that this is a result of: (1) adaptation of the transmission cycle to the peridomestic habitat because of urbanization and deforestation; (2) failure of vector and reservoir control campaigns; (3) emergence of *Leishmania* as an opportunistic infection in HIV-infected people and (4) increasing evidence of circulating, drug-resistant parasite strains. Economic and social pressures have led to deforestation of large parts of the tropics and, as a result, leishmaniasis

transmission cycles previously thought to be strictly sylvatic (e.g. *Leishmania braziliensis*) have now adapted to the peridomestic environment. For example, data from prospective, cross-sectional and retrospective epidemiological surveys have demonstrated that *L. braziliensis* transmission has increased tenfold in north-east Brazil during the past ten years, a period which coincided with deforestation of remnant rainforest for agriculture and settlements [1]. This trend has been paralleled by the number of studies reporting *Leishmania* infection in (peri)domestic animals and the observations that *Lutzomyia* vectors of *L. braziliensis* have increasingly become endophagic. Other epidemics were a result of collapses in the health infrastructure and the mass migration of susceptible individuals to leishmaniasis-endemic areas because of war, as recently evidenced in Sudan and, now Afghanistan and Pakistan (P. Desjeux, WHO, Geneva, Switzerland). Non-vector leishmaniasis transmission through, for example, blood transfusion or needle sharing amongst intravenous, HIV and *Leishmania* co-infected drug users (IVDUs), has become increasingly important. In southern Europe, it has been established that up to 70% of adult cases of visceral leishmaniasis (VL) are associated with HIV infection and up to 9% of all AIDS patients suffer from VL.

Using two different nested PCR-hybridization protocols, *Leishmania* DNA has been detected in 42% of syringes used by IVDUs collected from two different needle exchange programmes (J. Alvar, Instituto de Salud Carlos III, Madrid, Spain) [2]. These observations corroborated previous findings, demonstrating that *Leishmania* isolates from a leishmaniasis

outbreak among IVDUs were identical by genotype and zymodeme analysis.

Too many tools, but no gold standards

A battery of tools exists to diagnose leishmaniasis. PCR-based protocols have been used to detect *Leishmania* in samples from a range of different tissues from humans and dogs, but, when compared with standard serological tests, their use in mass screening of samples is limited because PCR-based methods are costly, cumbersome and lack sensitivity. A rapid, sensitive and specific diagnostic test, such as immuno-chromatographic dipstick tests, would be desirable for epidemiological mass-screening surveys (e.g. in dog culling or vaccination programmes). Although dipstick tests have proven to be sensitive and specific in detecting *Leishmania* infection in humans, a recent comparative study has shown that their sensitivity and specificity is variable when tested on dogs (L. Gradoni, Instituto Superiore de Sanità, Rome, Italy). Because parasitological diagnosis is characteristically insensitive, serological diagnosis – although unspecific – has remained the diagnostic choice for mass screening of dogs in epidemiological surveys. The advent of specific recombinant antigens has meant that the specificity of serological tests (e.g. ELISA) has been vastly improved. For example, several studies have shown that rK39, a *Leishmania donovani* complex-specific recombinant antigen, has high sensitivity and specificity [3], and allows the distinction between active, asymptomatic and cured infections.

Immunology and vaccines

Although effective in experimental animal models, killed *Leishmania* vaccines have