Table 1. Current interests and activities in *Eimeria* genomics worldwide*

<table>
<thead>
<tr>
<th>Country and current interests in <em>Eimeria</em> genomics</th>
<th>Associated website</th>
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</thead>
<tbody>
<tr>
<td>Brazil <em>Eimeria acervulina, Eimeria maxima</em> and <em>Eimeria tenella</em> ESTs; ORESTES; extrachromosomal genomes; bioinformatics</td>
<td><a href="http://www.ibm.fmwz.usp.br/">http://www.ibm.fmwz.usp.br/</a></td>
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<tr>
<td>China Whole genome sequencing of <em>E. maxima</em></td>
<td><a href="http://cgat.ukm.my/genomicslab/">http://cgat.ukm.my/genomicslab/</a></td>
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<tr>
<td>Malaysia Sequencing of chromosomes 1 and 2 of <em>E. tenella</em></td>
<td><a href="http://www.sanger.ac.uk/projects/e_tenella/">http://www.sanger.ac.uk/projects/e_tenella/</a> and <a href="http://www.iah.bbsrc.ac.uk/eimeria/index.html">http://www.iah.bbsrc.ac.uk/eimeria/index.html</a></td>
</tr>
<tr>
<td>UK <em>Eimeria tenella</em> whole genome shotgun sequencing and ESTs; <em>Eimeria</em> genome annotation; bioinformatics; proteomics of apical organelles; genetic linkage maps of <em>E. tenella</em> and <em>E. maxima</em></td>
<td><a href="http://www.mrc-lmb.cam.ac.uk/happy/happy-home-page.html">http://www.mrc-lmb.cam.ac.uk/happy/happy-home-page.html</a></td>
</tr>
<tr>
<td>USA <em>Eimeria acervulina</em> whole genome shotgun sequencing and ESTs</td>
<td><a href="http://www.anri.barc.usda.gov/pbel/index.asp">http://www.anri.barc.usda.gov/pbel/index.asp</a></td>
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*This list is not exhaustive, but provides a snapshot of the major projects on *Eimeria* genomics that are now ongoing. Abbreviations: EST, expressed sequence tag; ORESTES, open reading frame expressed sequences tags.

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**E. maxima**, two species that are also of great economic significance but which differ from *E. tenella* in many important aspects of their interactions with the chicken. Interest in these species has led to further expansion of the consortium (see Table 1). Detailed analysis of the genomes of *E. tenella*, perhaps with those of the other species, will be invaluable for developing an understanding of the biology and biochemistry of *Eimeria* parasites, for comparative analysis with other members of the Apicomplexa, and for guiding the selection of novel, effective targets for drug and vaccine design.

Acknowledgements

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References


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Research Focus

**Pregnancy-associated malaria – on the brink?**

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Regarded by many as the best example in falciparum malaria of an association between a specific parasite-adhesive phenotype and disease, pregnancy-associated malaria represents one of the more immediate hopes for control of malaria disease via vaccination. But, are we sure that we have identified the right candidate antigens and do we know how to measure the impact of such an intervention?

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www.sciencedirect.com
The impact of epidemic malaria during pregnancy was first described in 1937 [1]; since then it has been the subject of many descriptive studies [2]. The common finding of these studies was that pregnancy-associated malaria (PAM) was most frequent and severe in first pregnancies and that low-birth-weight (LBW), which is strongly associated with infant mortality [3], was a frequent outcome. Recent calculations (B. Brabin, pers. commun.) have indicated that as many as 300 000 fetal and infant deaths, and >2500 maternal deaths could be attributable to PAM every year, representing a major mortality risk.

At a recent meeting sponsored by the European Malaria Vaccine Initiative, a group of experts in the field reviewed our current knowledge of the biology of PAM and its impact on designing clinical trials for vaccines (http://www.envi.org/meetings.htm). In 1995, two articles were published that described chondroitin sulfate A (CSA) as a receptor for P. falciparum-infected erythrocytes (IE) [4, 5]. In 1996, Fried and Duffy showed that IE from placenta-bound to CSA (IECSA), but IE from non-pregnant donors did not [6], suggesting that the placenta could select a sub-population of IE that could bind to CSA and cause PAM. These findings gave rise to the hypothesis that the placenta can select for parasite variants that are not commonly found in children and non-pregnant adults in malaria-endemic regions. This explained both the lack of immunity seen in primigravid women and the reduction in susceptibility to PAM in subsequent pregnancies [7]. The past five years have produced a large body of published data supporting this suggestion, providing a biological framework to explain these observations, but they have also raised several questions on the biology of PAM.

Which var gene is expressed?

Of the several variant surface antigens on the IE, the most studied is Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1), which is encoded by a family of var genes. The evidence concerning which var gene is transcribed in parasites in infected pregnant women is conflicting. On the one hand, there are several papers that identify the var1csa [8–10] gene and define the CSA-binding region within this gene [11]. Antibodies raised against the corresponding recombinant protein label different IECSA and placental isolates [12], and can inhibit binding to cells expressing CSA on their surface [13]. On the other hand, since the discovery of the var2csa gene and the use of new primers to detect var expression, var2csa transcription has been shown to correlate with the CSA-binding phenotype [14], whereas var1csa is constitutively transcribed independent of the binding phenotype [15]. To complicate matters further, there is some evidence that other var genes [16, 17] and different regions within them [18] could mediate adhesion to CSA.

Why are IECSA associated with PAM not commonly found in paediatric malaria cases?

It has been clearly demonstrated that IECSA will adhere to endothelial cells under physiologically relevant flow conditions and CSA purified from these sources supports CSA-dependent binding [19]. If this is the case, then why are these types of parasite variants not common in children with malaria and why have adults not developed immunity against IECSA? The answer to this differential activation of var genes might lie in the distinct promoter regions associated with var1csa and var2csa genes [20], which might be switched on at very low rates (e.g. a switch rate of $1 \times 10^{-6}$ per generation) during antigenic variation, and the unusual structure and/or arrangement of these genes which acts to reduce the probability of other adhesive domains being inserted into them via recombination [21]. Research in this area has begun to blur the edges of these definitions: we know that IECSA are found in children (albeit at low levels) and IE non-CSA can be associated with PAM. Further work will be required to examine IE from placental and paediatric sources in more detail to understand the adhesive phenotypes seen in these variants, the role of PfEMP1 and var, and the contribution of other proteins in these interactions. We also have to address whether pregnancy itself governs the immune response to IECSA.

What is the mechanism of ring-stage IE adhesion?

The discovery of adherent ring-stage parasites indicates that some IE can be sequestered from the peripheral circulation for the entire asexual development cycle [22]. Why this property seems to be uniquely associated with IECSA is unknown. However, the identification of the rhoptry-associated protein 2 (RAP2) as a potential parasite ligand [23] has prepared the way for a detailed investigation into the behaviour of this protein in CSA-selected and -unselected IE. Further work is also required to examine the level of ring-stage adhesion in PAM because little or no binding of ring-stage parasites was found in one study [24].

PAM and possible control

Like other severe malarias, the pathology of PAM is not simple and includes stillbirth, perinatal, neonatal or infant mortality, and maternal anaemia [25–27]. There is no doubt that the burden of mortality seen in neonates, infants and mothers is significant [28]. The concentration of research on IE sequestration in the placenta raises the question as to whether a var CSAbased vaccine would be effective in controlling disease in mothers and in babies. Because pregnant women can control non-CSA-binding P. falciparum infections, it seems reasonable to suggest that such a vaccine would be effective in limiting maternal mortality and/or morbidity, in addition to having a significant effect on reducing LBW.

How does the parasite cause disease in pregnant women? Many studies have looked at infection in pregnancy only at the later stages of gestation, but pregnant women can frequently be chronically infected for several months. More work is needed to understand how different patterns of disease during pregnancy (e.g. chronic versus...
acute infection) affect outcome and how these relate to the gestational age at first infection. Even in the placenta, where we know that IE are sequestered, the actual processes causing pathology or the proportion of IE that are adherent are not known. How the changes in cytokine profiles alter intrauterine growth and prematurity has not been elucidated, nor the role of pregnancy in shaping disease.

Vaccines are very attractive for controlling disease in developing countries because of the long-term protection afforded, but we still do not know how many infections are necessary to induce parity-dependent immunity, which could have important implications for vaccine delivery. Other regimes have begun to show success, such as insecticide-treated nets (ITNs) and intermittent preventive treatment (IPT), although sustainable adequate coverage remains a formidable problem. For IPT, the shortage of safe drugs for use in pregnancy could become a major obstacle and so encouragement of an active drug development programme is an important component of PAM research.

Vaccines and animal models of PAM

Much of our knowledge on the molecular biology of PAM has come from studies using measurements of ex vivo adhesion and labelling of infected erythrocytes in PAM (IEPAM) with immune sera. While this has produced useful information linked to disease, it does not explain all the manifestations of the pathology of PAM. Several options for models exist and the best of these models involve primates. Not only would an animal model give access to samples that could not be obtained in humans, but it would also provide a platform for testing potential therapies (both for efficacy and safety).

In many ways, one of the easiest solutions to the var1csa and/or var2csa conundrum is to combine both antigens in a single vaccine. However, it is not known if the combination of antigens would lead to an inefficient or ineffective immune response, and this would have to be tested. Even if var1csa and var2csa are both involved in binding to CSA in the placenta, the involvement of other var genes or other genes cannot be ruled out, and more extensive sampling of wild parasite populations is needed to clarify this. Furthermore, the concentration of research in the IE-CSA component of PAM might also lead to problems if non-CSA-binding IE are able to take over the ‘compartment’ vacated because of the induction of immunity to IE-CSA by a vaccine. Current efforts towards a PAM vaccine are concentrating on the apparent conserved nature of the antigens presented on IEPAM.

It has been suggested that women before their first pregnancy would be an ideal target group for a PAM vaccine, but this raises questions on what age group should be vaccinated and how they could be recruited efficiently. Some of these women are likely to be pregnant, thus raising additional questions about immunogenicity and safety. Malaria in pregnancy is complex and could involve either chronic or repeated infection over months, or acute infection over a much shorter time span, which will have implications for the development of immunity. More work is needed to elucidate the development of immunity to IEPAM during pregnancy and the impact of these findings on vaccine delivery.

Conclusions

The fact that a PAM vaccine is even being discussed shows the rapid advances that this field has made over the past five years. The high levels of mortality and morbidity caused by PAM linked with the cross-reactivity seen in IEPAM establish the priority for a vaccine trial. The stage for clinical testing is approaching. However, at the point of moving into human trials, a new facet of the molecular biology of PAM has been discovered that needs to be integrated into our existing knowledge before taking the next step. Many questions remain, but the key question is whether to continue with a vaccine based on var1csa alone, or to consider a replacement with or the addition of var2csa? The scientific data appears to indicate that var2csa-type genes are commonly transcribed by IEPAM and are probably translated (A. Salanti, pers. commun.). However, there are data showing that var1csa can mediate binding to CSA, the principal receptor for IEPAM, and this gene is transcribed, albeit constitutively and in an unusual timeframe. It is possible that both classes of var genes are involved in PAM. The next logical step must be to use the new findings to reassess previous conclusions and to design new experiments, sharing reagents between laboratories, to produce definitive results to underpin the move into clinical testing.

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Mind the gap: bridging the divide between clinical and molecular studies of the trichomonads

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As is often the case in the study of infectious diseases, there are those interested in the basic biology of the organism and those primarily concerned with the clinical aspects of infection, disease and treatment. Although it is not uncommon for these two sets of researchers to remain essentially separate, the modern era of genomics is increasingly bringing them together. Such is the case with the community of scientists studying *Trichomonas vaginalis*, a human protozoan parasite of the urogenital tract that causes the sexually transmitted disease trichomoniasis.

*Trichomonas vaginalis* is the most prevalent sexually transmitted nonviral parasite found in North America, where it is responsible for ~5 million cases of trichomoniiasis annually [1]. Worldwide, over 170 million cases of trichomoniasis are reported each year [2]; for example, infection rates have been reported as high as 67% among patients attending a clinic in Mongolia [3] and 40% among indigenous Australian women in the Northern Territory [4]. In addition to its prevalence, infection with *T. vaginalis* is emerging as one of the most important cofactors in amplifying HIV transmission [5,6]. Infection with *T. vaginalis* is also recognized as contributing to low-birth-weight and preterm delivery [7]. A closely related parasite, *Tririchomonas foetus*, is the causative agent...