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A panel of recombinant proteins from human-infective *Plasmodium* species for serological surveillance

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Abstract

Background: Malaria remains a global health problem and accurate surveillance of *Plasmodium* parasites that are responsible for this disease is required to guide the most effective distribution of control measures. Serological surveillance will be particularly important in areas of low or periodic transmission because patient antibody responses can provide a measure of historical exposure. While methods for detecting host antibody responses to *Plasmodium falciparum* and *Plasmodium vivax* are well established, development of serological assays for *Plasmodium knowlesi*, *Plasmodium ovale* and *Plasmodium malariae* have been inhibited by a lack of immunodiagnostic candidates due to the limited availability of genomic information.

Methods: Using the recently completed genome sequences from *P. malariae*, *P. ovale* and *P. knowlesi,* a set of 33 candidate cell surface and secreted blood-stage antigens was selected and expressed in a recombinant form using a mammalian expression system. These proteins were added to an existing panel of antigens from *P. falciparum* and *P. vivax* and the immunoreactivity of IgG, IgM and IgA immunoglobulins from individuals diagnosed with infections to each of the five different *Plasmodium* species was evaluated by ELISA. Logistic regression modelling was used to quantify the ability of the responses to determine prior exposure to the different *Plasmodium* species.

Results: Using sera from European travellers with diagnosed *Plasmodium* infections, antigens showing species-specific immunoreactivity were identified to select a panel of 22 proteins from five *Plasmodium* species for serological profiling. The immunoreactivity to the antigens in the panel of sera taken from travellers and individuals living in malaria-endemic regions with diagnosed infections showed moderate power to predict infections by each species, including *P. ovale*, *P. malariae* and *P. knowlesi*. Using a larger set of patient samples and logistic regression modelling it was shown that exposure to *P. knowlesi* could be accurately detected (AUC = 91%) using an antigen panel consisting of the *P. knowlesi* orthologues of MSP10, P12 and P38.

Conclusions: Using the recent availability of genome sequences to all human-infective *Plasmodium* spp. parasites and a method of expressing *Plasmodium* proteins in a secreted functional form, an antigen panel has been compiled that will be useful to determine exposure to these parasites.

Keywords: *Plasmodium*, Serology, Antigen, Recombinant protein, Antibody, Malaria

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Background

Malaria is an infectious disease that remains a global health problem causing an estimated 219 million clinical cases resulting in 435,000 deaths in 2017 [1]. The disease

is caused by parasites of the genus *Plasmodium* and several species are known to regularly infect humans. The vast majority of deaths occur in sub-Saharan Africa and are caused by *Plasmodium falciparum*, whereas outside of Africa, *Plasmodium vivax* is responsible for over half of all malaria infections leading to significant morbidity and mortality [2]. Much less is known about the other human-infective *Plasmodium* species, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium knowlesi* both in terms of their global distribution and clinical impact. *Plasmodium knowlesi*, a parasite typically found in macaques, is a significant cause of human malaria in Southeast Asia, causing a spectrum of disease ranging from mild to fatal infections [3]. Malaysia has the highest incidence of *P. knowlesi* malaria with over 6700 cases reported in the last 2 years compared to only 85 cases of indigenous human malaria (unpublished data from the Ministry of Health, Malaysia).

Diagnosis of *Plasmodium* infections and epidemiological surveillance is important for guiding the distribution of resources into intervention measures and establishing their clinical impact over time [4]. Methods to measure the prevalence of *Plasmodium* infections include microscopy, rapid diagnostic tests (RDTs) and PCR-based approaches, each differing in their sensitivity, infrastructure requirements, and ability to diagnose the different species. Serological assays can provide a historical record of infection and because of the specificity of antibody-antigen binding, could also potentially discriminate between different *Plasmodium* spp. infections. Host antibodies appear rapidly after initial infection and can persist for months and even years after the parasites have been cleared [5, 6]. Serological screening has been applied in epidemiological settings to detect parasite exposure, evaluate transmission trends of malaria [7–10], and identify antibody-based correlates of protection [11, 12]. It is also used in blood donation centres, where, due to the increase in international travel and migration, the need for serological diagnosis is becoming more important to reduce the risk of transfusion-transmitted infections. Currently, many centres assess these risks using patient questionnaires which is generally unsatisfactory; moreover, the limitations and costs of the currently available serological tests often make implementing these assays economically unattractive [13].

Many antibodies recognise epitopes that are only formed in the context of an antigen in its native conformation [14]. To detect these antibodies, it is vitally important that the proteins used are correctly folded so that they faithfully form these epitopes. Expressing *Plasmodium* proteins in a soluble recombinant form has proved challenging, perhaps because of the high A:T content of the genome and lack of recognisable protein domains in many *Plasmodium* proteins [15]. This problem is especially acute for parasite proteins that are secreted or embedded in membranes because these proteins additionally contain structurally critical post-translational modifications, such as disulfide bonds that are not typically added by many commonly used expression systems. Recently, a method of expressing large panels of recombinant *Plasmodium* proteins was developed that retained many of their biochemical functions [16]. Central to this approach was the use of a mammalian expression system which increases the chances that appropriate post-translational modifications are correctly added to ensure proteins adopt their native fold. For antigens expressed using this method a large fraction—and in some cases all—the immunoreactivity to antigens was heat-labile, demonstrating that antibodies that recognise conformational epitopes represent a major component of the humoral response [16]. Previously, this approach was used to create libraries of soluble recombinant merozoite cell surface and secreted proteins that encompass the entire ectodomain from both *P. falciparum* [16, 17] and *P. vivax* [18]. Using sera from patients living in endemic regions, several of these proteins were found to be highly immunoreactive and might therefore be useful target antigens in serological assays [8, 12]. Expanding the antigen panel to include the other parasite species infecting humans would be especially valuable if they could be used to determine exposure to the different species of *Plasmodium*. Here, the recent availability of high-quality genome sequences from the three other human-infective *Plasmodium* parasites: *P. knowlesi*, *P. ovale* and *P. malariae* were used to extend the available panel of proteins and were tested for reactivity to sera from individuals infected with different *Plasmodium* parasites.

Methods

Study populations

Collection of sera from Malawian adults that were previously used to determine its effectiveness as an adjunct therapy to treat cerebral malaria was approved by the National Health Sciences Research Committee of Malawi [19]. Plasma from adult travellers, microscopy-diagnosed with malaria and species further confirmed by multiplex PCR for all species except *P. knowlesi* [20] after returning from visits to malaria-endemic regions were obtained from the Karolinska University Hospital, Stockholm, Sweden ($n=81$). Of these, 53 were from travellers of European origin and 28 from travellers born in malaria-endemic regions as follows: Angola (3 individuals), Burundi (1), Cameroon (1), Democratic Republic of the Congo (1), Eritrea (4), Ethiopia (1), The Gambia (1), India (3), Ivory Coast (2), Kenya (5), Pakistan (1), Thailand (1), and Uganda (4). Plasma from an endemic region with