A new window on *Plasmodium malariae* infections

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The distinctive clinical presentation, epidemiology and biology of the human malaria parasite *Plasmodium malariae* are not fully understood. The intra-erythrocytic cycle is 72 hours in duration, and so episodes of fever are typically 3 days apart, this being the reason for the historical term “quartan malaria”. The illness is usually treatable, although rare cases of fatal nephritis are well described in the literature [1, 2]. Remarkably, symptomatic episodes can present years or decades after exposure to *P. malariae* infection, and this species is widely acknowledged to be the Olympic champion of persistence in human hosts [3-5]. This persistence underlies an apparent discrepancy in estimates of prevalence of *P. malariae* worldwide. Compilations of species-specific malaria data from non-endemic countries with a heavy burden of imported cases, including the UK, China, Italy and France, consistently show *P. malariae* to be the least frequent cause of primary malaria attacks, with significantly fewer cases than either *P. ovale curtisi* or *P. ovale wallikeri* [6-10]. This implies that the mosquitoes transmitting malaria to visitors of endemic countries are less likely to carry *P. malariae.*  In contrast, species-specific data from field surveys of human peripheral blood samples just as consistently show *P. malariae* to bemore prevalent than *P. ovale* spp. in endemic settings, usually occurring in mixed species infections with *P. falciparum* or *P. vivax* [11-14]. This is most likely a direct result of *P. malariae* infections being acquired less often than *P. ovale* spp. but remaining in the blood at detectable levels for much longer. Finally, the assembly of genome sequence datasets for *P. malariae* has enabled researchers to identify unexpected adaptations in this species [15, 16]. Of particular interest is the expansion of gene families encoding variant antigens, including the *fam-l* and *fam-m* sub-families, which have no direct orthologues in other members of the genus and are distributed through the genome as co-expressed sub-telomeric pairs [5, 16]. The function of these expanded gene families is not yet known. There is thus a long list of questions regarding *P. malariae* for which we do not yet have answers, but published work on this parasite is scarce due to it being relatively inconspicuous in the field, rarely associated with severe complicated malaria [2] and not being cultivatable *in vitro.*

One of the most important questions about *P. malariae,* with its quartan erythrocytic cycle, is its relative susceptibility to antimalarial drugs compared with other species. Well documented evidence from a small number of African studies [12, 13] and one imported case [17] suggests an ability to survive ACT treatment in a subset of hosts. There is also evidence that, in some exposed individuals, *P. malariae* can break through otherwise effective chemoprophylaxis such as atovaquone-proguanil, a phenomenon that seems to be linked to late onset of symptoms in returning travellers [4]. It has been postulated that there may be unique features of *P. malariae* biology rendering it intrinsically less susceptible to some drugs and that the 72-hour life cycle in particular means survival following a 3-day course of a short half-life compound such as artemisinin is more likely [4,5]. This is a likely explanation for the inability of therapeutic drugs to completely clear all infections, but a slower, more extended schizogony period of 10-16 days in the pre-erythrocytic hepatic stage may also assist in evading prophylaxis. Another proposal is that genetic diversity among *P. malariae* populations may generate a range of drug susceptibilities to commonly used antimalarials [17]. In this context, it is of course important to remember that co-endemicity with *P. falciparum* and *P. vivax* across its geographical range means that populations of *P. malariae* will have experienced substantial drug pressure since the widespread deployment of quinine began in the 1820s. It is therefore possible that some drug resistance mutations are present in current populations of *P. malariae*. However, the extended intra-erythrocytic cycle is likely to remain the most important contributor to observed *P. malariae* persistence / recrudescence after treatment with dihydroartemisinin-piperaquine [13] or artemether-lumefantrine [17]. It can thus be argued that 6-day ACT regimens should be tested as a means to ensure complete clearance of *P. malariae* infections in all cases, as has been suggested for *P. falciparum* [18]. However, there are few opportunities to test such regimens in the field, as enrolment of sufficient *P. malariae* cases, without co-infecting *P. falciparum* or *P. vivax* being present would be a major challenge.

Given the many questions we have about *P. malariae*, and the paucity of studies dedicated to this interesting and ubiquitous parasite, the study of Woodford and colleagues in the current issue of *The* *Journal of Infectious Diseases* is very welcome [19]. These authors describe the development of a new induced blood-stage malaria model, in which volunteers are inoculated intravenously with an aliquot of *P. malariae-*infected erythrocytes originally cryo-preserved from peripheral blood donated by a patient in Brisbane, Queensland, who had travelled to Guinea-Conakry in 2013. The genome of this isolate has been previously sequenced [16].Two adult male volunteers participated in the trial, and both developed qPCR-detectable peripheral parasitaemia on day 7 post-inoculation. Onset of symptoms occurred on day 21 and day 25, at which point both direct and indirect (via membrane) mosquito feeding was performed, samples for measuring biomarkers and parasite transcription were taken and treatment with artemether-lumefantrine initiated. The primary endpoint of this study was safety, and neither volunteer experienced anything more than standard uncomplicated malaria symptoms, and the irritation of mosquito bites. There were no indications of the most serious clinical manifestations of *P. malariae* infections, anaemia and nephritis [2], which are both most likely to occur in children with heavy or chronic infection. Treatment initiation was triggered by appearance of symptoms, and both individuals made a complete recovery, remaining parasite-free at 3 months of follow-up. The authors were able to confirm infectiousness to mosquitoes of one subject, as evidenced by one oocyst on the midgut of one of 34 mosquitoes which fed directly on the skin. Nevertheless this is new information – even a very acute *P. malariae* infection can generate viable gametocytes that are infective to *Anopheles.*

A number of useful pilot investigations were carried out in this first trial, and these will help define important questions to be addressed in future work. The parasite clearance half-life immediately following initiation of treatment, an established indicator of the artemisinin kill-effect, was estimated at 6.7 hours, being similar for the two subjects. This is substantially longer than the cut-off of 5 hours considered as the upper-limit for adequate artemisinin susceptibility in *P. falciparum,* and thus provides additional support for the notion that *P. malariae* may indeed be intrinsically less susceptible to artemisinin than is *P. falciparum* [13]. Longer regimens could therefore provide better ACT efficacy against *P. malariae* and need to be tested. The transcriptomic analysis reported by Woodford *et al.* was dogged by the expected problems of poor depth and breadth of read coverage due to the relatively low parasite densities available; after removing reads mapping to host, PhiX and parasite ribosomes, 3.8 million informative reads remained, representing 1.5% of the total output. Despite these difficulties, the preliminary observations based on these data will inform transcriptional analyses going forward. The most startling finding was the complete lack of evidence of transcription from any of the hundreds of *fam-m* and *fam-l* gene pairs, which are postulated to be expressed together to form heterodimers [16]. This unexpected outcome needs further investigation, but *a priori* it may be that mRNA from this family is at very low abundance and thus below stringency cut-offs in the present analysis, very tightly controlled at a specific stage in the erythrcoytic cycle or not expressed in blood stages at all.

The new induced blood-stage model of *P. malariae* infection in humans opens a new window onto this species. The proof-of principle provided by Woodford *et al.* is a small study (N = 2), but represents an advance that should provide the opportunity for new studies of vaccinology, therapeutics and diagnostics for this species, as well as detailed investigations into *P. malariae* biology.

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