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Plasmodium malariae and *Plasmodium ovale* – the 'bashful' malaria parasites

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Abstract

Although *Plasmodium malariae* was first described as an infectious disease of humans by Golgi in 1886 and *Plasmodium ovale* identified by Stevens in 1922, there are still large gaps in our knowledge of the importance of these infections as causes of malaria in different parts of the world. They have traditionally been thought of as mild illnesses that are caused by rare and, in case of *P. ovale*, short-lived parasites. However, recent advances in sensitive PCR diagnosis are causing a re-evaluation of this assumption. Low-level infection seems to be common across malaria-endemic areas, often as complex mixed infections. The potential interactions of *P. malariae* and *P. ovale* with *Plasmodium falciparum* and *Plasmodium vivax* might explain some basic questions of malaria epidemiology, and understanding these interactions could have an important influence on the deployment of interventions such as malaria vaccines.

Geographical distribution

Although distribution of *Plasmodium malariae* infection is reported as being patchy, it has been observed in all major malaria-endemic regions of the world [1]. *P. malariae* infections are most common in sub-Saharan Africa and the southwest Pacific, where age-specific prevalence in mass blood surveys have exceeded 15–30% [2–8]. By contrast, when *P. malariae* has been detected in malaria-endemic regions of Asia [9–12], the Middle East [13], South America [14] and Central America [15], it is observed as an infrequent infection, with blood-smear light microscopy (LM) prevalence rarely exceeding 1–2%. Much higher levels of infection were, however, found in montagnard refugees from the Cambodian–Vietnamese border [16]. In South America, *P. malariae* is thought to be a zoonotic infection because the genetically identical *Plasmodium brasilianum* infects new-world monkeys [17] and both monkeys and humans in endemic areas show high levels of seropositivity to *P. malariae* and *P. brasilianum* antigens [18].

Plasmodium ovale was thought to have a much more limited distribution, with endemic transmission traditionally described as being limited to areas of tropical Africa, New Guinea, the eastern parts of Indonesia and the Philippines [19,20]. Infections with *P. ovale*, however, have also been reported in the Middle East [13], the Indian subcontinent [21] and different parts of Southeast Asia [11,22,23]. In West Africa (and to a lesser extent Central Africa), age specific LM prevalence of >10% have been observed [3,6]. However, in most

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places where *P. ovale* is observed, it is relatively uncommon and its prevalence (as detected by LM) rarely exceeds 3–5% [22,24–26].

Indepth descriptions of the epidemiology of both infections based upon LM data are, thus, restricted almost exclusively to highly endemic areas in Africa and the Southwest Pacific. Detailed epidemiological studies from South America and Asia are lacking.

Variation in parasite prevalence

In West Africa, *P. malariae* prevalence has been reported to peak at ages similar to those of *P. falciparum* (i.e. in children under ten years of age) [3,6,8]. In children under four years, in addition to adults, most LM-diagnosed infections were observed to be of low density (<500 parasites per μl) and, at least in children, usually of long duration [6]. Coinfections of *P. malariae* and *P. falciparum* were reported more commonly than would be expected by chance [3,27,28]. Overall, *P. malariae* has been characterized to exhibit opposing seasonal fluctuation with *P. falciparum*, with *P. malariae* prevalence [8,26] and/or parasite densities [5] increasing in the dry season. A high incidence of new *P. malariae* infections in young, nonimmune children reported during the rainy seasons [8] led Molineaux and Grammiccia to suggest that seasonal fluctuation in the *Plasmodium* species infection formula resulted from suppression of *P. malariae* by frequent *P. falciparum* infection during the rainy season rather than variation among factors that could lead to differences in parasite transmission [8].

Studies from Papua New Guinea (PNG) indicate that LM peak prevalence of *P. malariae* infection might be similar to those observed in West Africa (i.e. 15–30%); in PNG, however, *P. malariae* infection is observed predominantly in older children (seven to nine years) and adolescents (ten to 16 years) [4,25,29]. In addition, *P. malariae* infection prevalence peaked in noticeably later age groups than did *P. falciparum* infections [30]. Outside Africa there has been no report of opposing seasonal fluctuation in the prevalence of *P. malariae* and *P. falciparum* infections. Moreover, mixed infections are either randomly distributed [31,32] or there might be a short age of LM-detected mixed infections [28]. Apart from differences in a wide range of ecological factors (such as climate and Anopheline vector species), these contrasting observations in *P. malariae* epidemiology could be linked to the virtual absence of *Plasmodium vivax* infections in many African populations because of the lack of the Duffy blood-group-antigen, which *P. vivax* requires to invade red blood cells [33]. Interestingly, Smith *et al.* reported that in Wosera, PNG, *P. vivax* infections might be associated with an antagonistic effect on *P. malariae* infections [34].

Like *P. malariae, P. ovale* infections in West African populations tend to be most common in children under ten years of age [3,6,35]. The low numbers of LM detectable infections in most PNG and Southeast Asian surveys precludes a firm assessment of age distribution of infections in these regions. Indepth studies of *P. ovale* in Senegal reported that, in all age groups, most *P. ovale* infections were patent (i.e. detectable by LM) for fewer than two weeks and maximum parasitemia rarely reached levels that were sufficient to introduce clinical attacks (i.e. <800 parasites per μl) [6,35]. However, the incidence of infections was high, with 40% of people of all ages experiencing at least one *P. ovale* infection during the four-month study period [6,35]. The rarity of reported *P. ovale* infections in cross-sectional surveys might be heavily influenced by the low species-specific parasitemia and short duration of patent infections.

Clinical malariae and ovale malaria

Although *P. malariae* and *P. ovale* infections are common and pyrogenic thresholds likely to be lower than in *P. falciparum* [36], the incidences of clinical *P. malariae* and *P. ovale* episodes are low [6,25] and might account for only 1–2% of fever episodes [36]. In areas with marked variation in seasonal climate, *P. malariae* might contribute to 50% of malaria episodes in the low-transmission season [37]. Because differentiation between *P. malariae* and *P. falciparum* is difficult by thick-film microscopy, the incidence of clinical *P. malariae* could, however, often be underestimated.

Despite a recent report of chloroquine (CQ) resistant *P. malariae* in Indonesia [38], *P. malariae* and *P. ovale* remain highly sensitive to CQ and other common antimalarials [39] and might, thus, be effectively killed even by residual drug levels [40]. In addition, given their slower development in the mosquito [41], *P. malariae* and to a lesser extent *P. ovale* might be susceptible to interventions that are aimed at reducing transmission. In a study performed in Burkina Faso, insecticide-treated bednets were associated with a greater reduction of *P. malariae* prevalence than of *P. falciparum* [42]. Similarly, in PNG, *P. malariae* prevalence was strongly reduced by both indoor residual dichloro-diphenyl-trichloroethane (DDT) spraying and mass drug administration and, unlike *P. falciparum, P. malariae* failed to recover to precontrol levels after DDT spraying was stopped [43,44].

Most of what is known with regard to the epidemiology of *P. malariae* and *P. ovale* has been shaped by malariometric surveys based upon LM diagnosis, and this has certainly influenced the studies described here so far. Unfortunately, differentiation of *P. malariae* from *P. falciparum* and *P. ovale* from *P. vivax* by LM can be challenging [45]. Low parasitemias, which are commonly observed for *P. malariae* and *P. ovale*, require evaluation of thick-films; however, this technique results in the loss of many distinguishing features of infected red blood cells. Therefore, in endemic regions where *P. falciparum* and/or *P. vivax* predominate, *P. malariae* and *P. ovale* are frequently overlooked. For more accurate diagnosis and estimates of the burden of *P. malariae* and *P. ovale* infections, more sensitive diagnostic methods are needed.

Improved diagnosis of *P. malariae* and *P. ovale* infections by PCR

Although acridine orange microscopy substantially improves detection levels over Giemsa thick-film LM [11,46], PCR-based diagnostic methods targeting the small subunit rRNA (SSU rRNA) gene [47] have transformed perspectives on malaria epidemiology [48]. These assays enable detection of the four *Plasmodium* parasite species that infect humans at densities ~100 times lower than the limit of LM detection [47] and have evolved from simple species-specific amplification strategies to multiplex approaches that incorporate semiquantitative assessments [31,49–56]. We have greatly extended the potential use of PCR diagnosis for a wide range of epidemiological studies through our recent efforts to develop a ligase detection reaction fluorescent microsphere assay (LDR–FMA) [52,57] to evaluate all four malaria parasites of humans in a single-well multiplex format.

In a series of studies from PNG that have combined LM and molecular diagnostics [29,31,32], we have found that molecular methods consistently detected significantly increased prevalence of all four malaria species of humans, with the largest increases in *P. malariae* and *P. ovale*. In these studies from three different PNG populations, the prevalence of *P. falciparum* and *P. vivax* increased between 1.6 and 3.0-fold and 2.1 and 3.4-fold, respectively, and a 2.6 to 10.9-fold increase in *P. malariae* prevalence was observed (LM: 1.1–14.4%, PCR: 10.0–37.0%) (Table 1). Even larger increases in prevalence were observed for *P. ovale.* Detection of *P. ovale* by LM ranged from 0 to 0.3%, whereas LDR–FMA detected *P. ovale* infection in 5.2 to 15.6% of the study subjects. Overall, the majority of *P.*

malariae and *P. ovale* diagnoses have been observed in the context of mixed infections with *P. falciparum* and/or *P. vivax*, including several quadruple infections [29,31,32]. Therefore, it is likely that *P. malariae, P. ovale* and mixed species infections are substantially more common in PNG than previously thought. The direct comparison between LM and LDR–FMA diagnosis of *P. malariae* infection for 1182 individual samples showed that, although we observed an overall concordance between diagnostic methods of 89.1%, there was a wide variation between LM-based parasitemia and the semiquantitative LDR–FMA fluorescent signal [29]. These findings indicate that there are difficulties with LM in detecting parasites across wide-ranging levels of blood-stage infection. Similar observations were made for *P. falciparum* and *P. vivax* diagnoses [29].

Comparisons between infection prevalence and age can now be optimized because of the increased ability of molecular diagnostic assays to evaluate samples from large numbers of study participants with greater specificity and sensitivity. Results presented in Figure 1 show a shift of the burden of infections into older age groups for PCR detectable infections compared with diagnosis by LM [29]. In all species except *P. vivax*, in which infections were most commonly found in children seven to nine years old (42.6%), prevalence of infections peaked in the adolescent age group (ten to 19 years), which had an overall infection rate of 69.9%, *P. falciparum* = 48.6%, *P. malariae* = 20.9%, *P. ovale* = 9.3% and mixed infections = 25.7%. Further studies in other populations will be needed to confirm this observation.

Consistent with our observations in PNG, when patients have been studied using PCR-based methods in Africa, Southeast Asia and Brazil, similar large increases in *P. malariae* and *P. ovale* prevalence has been detected (Table 1). In two African studies, PCR prevalence of *P. malariae* was 23.3% and 39.2%, and *P. ovale* prevalence was 6.9% and 9.3%. By contrast, LM detected no non-*P. falciparum* infections in the first study [58] and 18.7% *P. malariae* and 2.8% *P. ovale* in the second [59]. Findings consistent with these observations have been generated recently in a study of >1200 Kenyans. Preliminary comparisons between LM and LDR–FMA diagnostic methods indicate that, although LM detected no non-*P. falciparum* infections, LDR–FMA estimated prevalence of *P. malariae* to be 13.5% and of *P. ovale* to be 3.1% (C.H. King and P. Zimmerman, unpublished). PCR diagnosis detected similarly high rates of infections in a rural village in Nigeria (*P. malariae* = 26.1%, *P. ovale* = 14.8% [60]) and in pregnant women in Kenya (*P. malariae* = 24.5%, *P. ovale* = 22.5% [61]). However, in a study in southern Mozambique that typed infections by PCR, substantial spatial and seasonal variations in the prevalence of *P. malariae* were observed [62]. In the Thai–Myanmar border area, up to 25% of malaria patients were observed to carry subpatent *P. malariae* infections, whereas 4% showed evidence of subpatent *P. ovale* infections [46]. These observations were repeated in many other Southeast Asian settings, with an average of 16.6% and 3.5% of malaria patients harbouring *P. malariae* and *P. ovale* parasites, respectively [11]. This confirmed that both parasite species are common across much of the region. In the Brazilian Amazon, *P. malariae* prevalence of 11.9% in the general population [14] and 9.4% in patient samples were detected by PCR [63]. As in PNG, the majority of infections were found in the presence of *P. falciparum* and/or *P. vivax* (Table 1).

Although there are an increasing number of studies that use PCR diagnosis of *P. malariae* and *P. ovale* infections in human blood samples, the diagnosis of sporozoite infections in mosquitoes still relies almost exclusively on ELISA-based dectection of circumsporozoite protein (CSP) [64]. Unfortunately, recent analysis of the available CSP ELISA showed the test to have insufficient sensitivity to detect infections with low numbers of *P. malariae* sporozoites [65]. Beier *et al.* indicate that low sensitivity of the ELISA method is unlikely to be restricted to *P. malariae* but also apply to other malaria species, including *P. falciparum* [66]. Therefore, PCR diagnosis of mosquito salivary glands will be invaluable for accurate

quantification of the prevalence of *P. malariae* and *P. ovale* sporozoite infections. A recent study in Guinea–Bissau [67] that used PCR on salivary glands and/or midgut oocysts from blood-fed resting mosquitoes found 1–5% and 0.4–20% to be infected with *P. malariae* and *P. ovale*, respectively, whereas another study from Mozambique found 5% of all positive mosquitoes to be infected with *P. malariae* [62].

One note of caution regarding current PCR-based reports on *P. malariae* and *P. ovale* prevalence is that the sequence variation in the SSU rRNA gene target of both species must be considered [68–70]. From studies in Southeast Asia, 27% of *P. malariae* and 36% of *P. ovale* infections were due to single variant strains in each species that have not been included in all PCR-based diagnostic assays [11]. To date, variants of this nature have not been observed in PNG. In addition, it should be noted that although the sensitivity of PCR is superior to LM even PCR will fail to detect extremely low-level infections.

On the best available evidence, it is probable that *P. malariae* is a common infection to all malaria-endemic areas, whereas *P. ovale* might be present at 2% to >10% prevalence in the general population of Africa and New Guinea and among malaria patients in Southeast Asia. Both large increases in prevalence and high complexity of infections indicate that PCR-based diagnosis has to be the standard in future studies of the epidemiology of *P. malariae* and *P. ovale*.

Why are *P. malariae* and *P. ovale* infections still important?

Given that both *P. malariae* and *P. ovale* are relatively mild infections and are easily curable with common antimalarials, why should we pay closer attention to this increased burden of infections with either species?

First, considering the problems with LM diagnosis, the actual burden of illness could be markedly underestimated and PCR diagnosis studies of clinical cases are thus needed. Although considered mild, *P. malariae* can cause a chronic nephrotic syndrome that, once established, does not respond to treatment and carries a high rate of mortality [71]. In addition, *P. malariae* is known to cause chronic infections that can last for years [39] and might reoccur decades after initial exposure when people have long since left endemic regions [72]. The health burden of such chronic or reoccurring infections in an endemic context is not clear.

Second, some studies based on LM indicate that there is evidence for interactions between *P. malariae*, and possibly *P. ovale*, with *P. falciparum* and *P. vivax* infections. Although cross-sectional studies have reported positive associations between infections of *P. falciparum* and *P. malariae* and/or *P. ovale*, respectively [3,27,28,34], these associations are more likely to represent individual differences in exposure (i.e. children with a high risk of acquiring *P. falciparum* infections also have a high risk of acquiring other infections) [34] or susceptibility to infection [27], rather than true biological interactions between the parasite species.

Third, because *P. malariae* is often overlooked by LM, it is difficult to assess potential negative associations or ‘suppression’ by *P. falciparum* and/or *P. vivax* in mixed infections. The observed seasonal differences in Africa between *P. falciparum* and *P. malariae* prevalence rates and *P malariae* densities have been interpreted as a suppression of *P. malariae* during the periods of high *P. falciparum* transmission, at least in older individuals [5,8,27]. In addition, in PNG a comparatively later age of peak prevalence of *P. malariae*, seven to 16 years [4,25,29], is observed, compared with that in Africa, under ten years [3,6,8]. Does this observation indicate a negative interaction between *P. vivax* (which is most common in children under ten years) and *P. malariae*?

Most importantly, however, is the possibility that *P. malariae* infections might have a mitigating effect on both *P. falciparum* and non-*P. falciparum* illness and, therefore, that treatment of *P malariae* and/or *P. ovale* could increase the pathogenesis that is associated with other malaria species. In Nigeria, Black *et al.* [73] observed (by PCR) a significantly lower prevalence of coinfections with *P. malariae* in clinical *P. falciparum* cases (0%) compared with asymptomatic controls (27%). They hypothesized that chronic infections with *P. malariae* could contribute to a downregulation of the cytokine cascade. In another African study, α-thalassaemic pregnant women had a higher risk of harbouring mixed *P. malariae* and *P. falciparum* infections but a lower risk of febrile symptoms and signs of inflammation than women who were infected with *P. falciparum* alone [74]. This led the authors to propose that the increased susceptibility to *P. malariae* might partly be responsible for the mild courses of *P. falciparum* malaria that occur in α-thalassaemic pregnant women. Similarly, in a study in PNG, infections with *P. malariae* were associated with a subsequent decrease in overall health-centre attendance with presumptive malaria, with a stronger effect on non-*P. falciparum* rather than *P. falciparum* disease [34]. By contrast, observations from the Gambia showed that in children under seven years *P. malariae* episodes are most common in the dry season, when *P. falciparum* infections and illness are less common [37]. This indicates that suppression of *P. malariae* by *P. falciparum* might account for the low level of morbidity associated with *P. malariae* infections in African children. Understanding the potential for such complex interactions on the morbidity that is attributable to each of the malaria species of humans could be complicated by inaccurate diagnosis.

Little is known about the potential for interactions between *P. ovale* and other malaria infections. However, the fact that *P. ovale* has been found to be most prevalent in areas of West Africa, where *P. vivax* is almost absent because of the high prevalence of the Duffy blood-group-negative phenotype [33], might also indicate a negative interaction between these two species. Because *P. ovale* prevalence and parasitemia are consistently low, it is often the case that there are insufficient observations to enable meaningful statistical evaluations regarding interactions between *P. ovale* and the other malaria parasite species that infect humans.

Concluding remarks

With vaccines being developed against *P. falciparum* and *P. vivax*, it is important to determine the burden of infection and disease due to *P. malariae* and *P. ovale* and assess the potential for interaction between these malaria-parasite species. Should negative interactions be important, then reducing the burden of *P. falciparum* or *P. vivax* morbidity could increase the burden of morbidity that is attributable to *P. malariae* and *P. ovale*. The possibility for such an effect is highlighted by studies in Brazil and PNG that found high levels of *P. malariae* infections in isolated populations where either *P. falciparum* was absent [75] or *P. vivax* rare [76]. Further studies, using PCR diagnosis, into the burden of infection and illness with *P. malariae* and *P. ovale* in different parts of the world are, therefore, clearly warranted.

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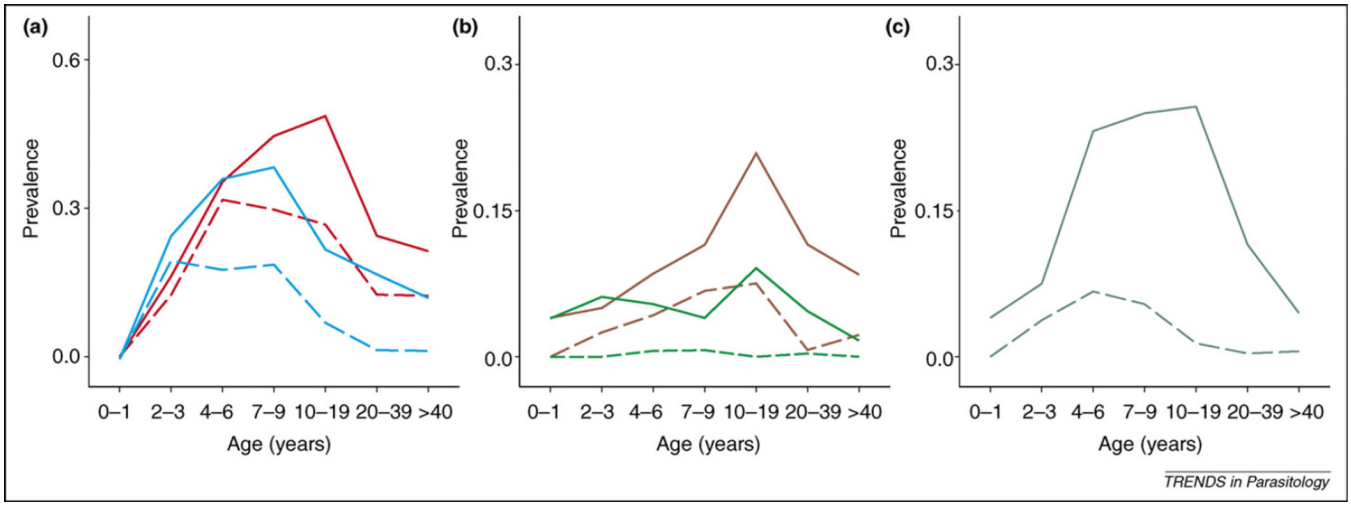

Figure 1.

Age-specific prevalence of *Plasmodium* infections. **(a)** *Plasmodium falciparum* (red) and *Plasmodium vivax* (blue) infections. **(b)** *Plasmodium malariae* (brown) and *Plasmodium ovale* (green) infections. **(c)** Mixed infections: concurrent infection with more than one *Plasmodium* species. Infections were detected by light microscopy (broken line) and LDR–FMA assay (unbroken line) in 1182 paired samples from Wosera, PNG [29].

Table 1

Studies comparing LM and PCR diagnosis of *Plasmodium malariae* and *Plasmodium ovale* infections

Region and country	Population	*P. malariae* LM (%)	PCR (%)	Mixed[a] (%)	*P. ovale* LM (%)	PCR (%)	Mixed[a] (%)	Refs
Pacific								
PNG (Drikikir)	CS[b], all ages	14.4	37.0	91.8	0.0	15.6	100.0	[31]
PNG (Liksul)	CS, all ages	1.1	10.0	75.8	0.0	5.2	88.2	[32]
PNG (Wosera)	CS, all ages	2.0	21.8	72.9	0.1	6.2	80.0	[32]
PNG (Wosera)	CS, all ages	4.0	12.4	69.3	0.3	5.5	77.3	[29]
Africa								
Cameron	CS, pregnant women	1.1	7.6	100.0	0.0	2.5	100.0	[77]
Equatorial Guinea	CS, under six years old	18.7	39.2	92.9	2.8	9.3	60.0	[59]
Guinea–Bissau	CS, all ages	0.0	23.3	97.7	0.0	6.9	100.0	[58]
Americas								
Brazil	CS, all ages	1.2	11.9	69.5	_[c]	–	–	[14]
Brazil	Malaria patients	0.0	9.4	88.9	–	–	–	[63]
Asia								
Thai–Myanmar border	Patients	2.2	24.3	99.2	0.4	3.8	100.0	[46]

[a] Proportion of *P. malariae* or *P. ovale* infections diagnosed by PCR that are coinfections with *P. falciparum* and/or *P. vivax*.

[b] Cross-sectional population survey.

[c] – indicates not assayed.